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(54) Title: MODULAR DESIGN AND SYNTHESIS OF OXAZOLONE-DERIVED MOLECULES

(57) Abstract

The design and synthesis of novel oxazolone-derived molecular modules and the use of the modules in the construction of new molecules and fabricated materials is disclosed. The new molecules and fabricated materials are molecular recognition agents useful in the design and synthesis of drugs, and have applications in separations and materials science.

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MODULAR DESIGN AND SYNTHESIS OF OXAZOLONE-DERIVED MOLECULES

1. FIELD OF THE INVENTION

The present invention relates to the *logical* development of biochemical and biopharmaceutical agents and of new materials including fabricated materials such as fibers, beads, films, and gels. Specifically, the invention relates to the development of molecular modules derived from oxazolone (azlactone) and related structures, and to the use of these modules in the assembly of simple and complex molecules, polymers and fabricated materials with tailored properties; where said properties can be planned and are determined by the contributions of the individual building modules. The molecular modules of the invention are preferably chiral, and can be used to synthesize new compounds and fabricated materials which are able to recognize biological receptors, enzymes, genetic materials, and other chiral molecules, and are thus of great interest in the fields of biopharmaceuticals, separation and materials science.

2. BACKGROUND OF THE INVENTION

The discovery of new molecules has traditionally focused in two broad areas, biologically active molecules, which are used as drugs for the treatment of life-threatening diseases, and new materials, which are used in commercial, especially hightechnological applications. In both areas, the strategy used to discover new molecules has involved two basic operations: (i) a more or less random choice of a molecular candidate, prepared either via chemical synthesis or isolated from natural sources, and (ii) the testing of the molecular

candidate for the property or properties of interest. This discovery cycle is repeated indefinitely until a molecule possessing the desirable properties is located. In the majority of cases, the molecular types chosen for testing have belonged to rather narrowly defined chemical classes. For example, the discovery of new peptide hormones has involved work with peptides; the discovery of new therapeutic steroids has involved work with the steroid nucleus; the discovery of new surfaces to be used in the construction of computer chips or sensors has involved work with inorganic materials, etc. As a result, the discovery of new functional molecules, being ad hoc in nature and relying predominantly on serendipity, has been an extremely time-consuming, laborious, unpredictable, and costly enterprise.

A brief account of the strategies and tactics used in the discovery of new molecules is described below. The emphasis is on biologically interesting molecules; however, the technical problems encountered in the discovery of biologically active molecules as outlined here are also illustrative of the problems encountered in the discovery of molecules which can serve as new materials for high technological applications. Furthermore, as discussed below, these problems are also illustrative of the problems encountered in the development of fabricated materials for high technological applications.

2.1 Drug Design

Modern theories of biological activity state that biological activities, and therefore physiological states, are the result of molecular recognition events. For example, nucleotides can form complementary base pairs so that complementary single-stranded molecules hybridize resulting in double- or triple-helical structures that appear to be involved in regulation of gene expression. In another example, a biologically active molecule, referred to as a ligand, binds with another molecule, usually a macromolecule referred to as ligand-acceptor (e.g. , a receptor or an enzyme), and this

binding elicits a chain of molecular events which ultimately gives rise to a physiological state, e.g., normal cell growth and differentiation, abnormal cell growth leading to carcinogenesis, blood-pressure regulation, nerve-impulse-generation and -propagation, etc. The binding between ligand and ligand-acceptor is geometrically characteristic and extraordinarily specific, involving appropriate three-dimensional structural arrangements and chemical interactions.

2.1.1 Design and Synthesis of Nucleotides

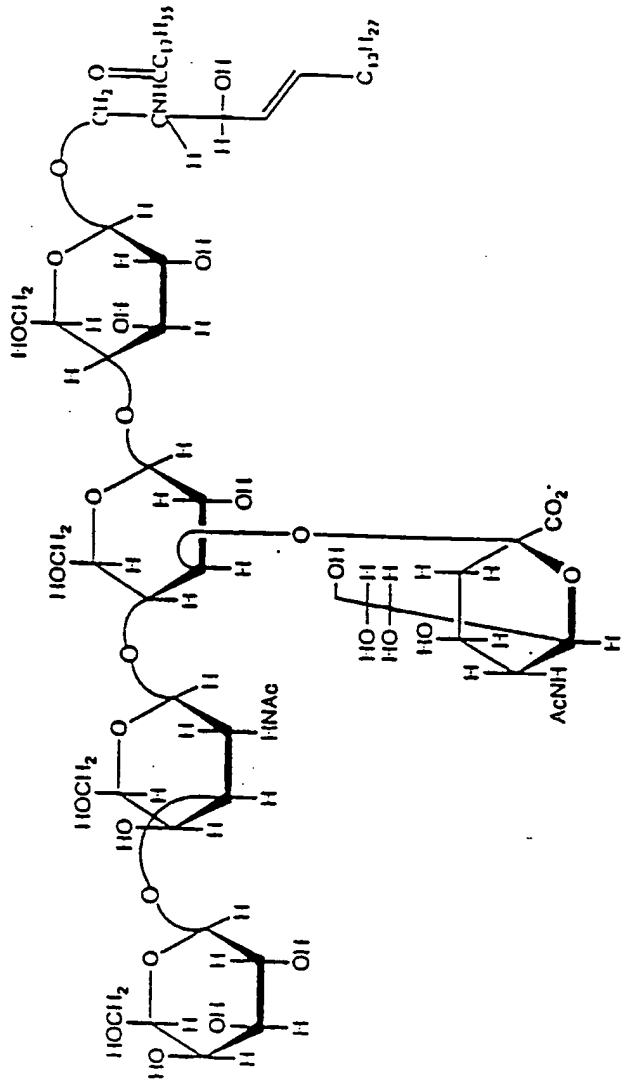
Recent interest in gene therapy and manipulation of gene expression has focused on the design of synthetic oligonucleotides that can be used to block or suppress gene expression via an antisense, ribozyme or triple helix mechanism. To this end, the sequence of the native target DNA or RNA molecule is characterized and standard methods are used to synthesize oligonucleotides representing the complement of the desired target sequence (see, S. Crooke, The FASEB Journal, Vol. 7, Apr. 1993, p. 533 and references cited therein). Attempts to design more stable forms of such oligonucleotides for use in vivo have typically involved the addition of various groups, e.g., halogens, azido, nitro, methyl, keto, etc. to various positions of the ribose or deoxyribose subunits (cf., The Organic Chemistry of Nucleic Acids, Y. Mizuno, Elsevier Science Publishers BV, Amsterdam, The Netherlands, 1987).

2.1.2 Glycopeptides

As a result of recent advances in biological carbohydrate chemistry, carbohydrates increasingly are being viewed as the components of living systems with the enormously complex structures required for the encoding of the massive amounts of information needed to orchestrate the processes of life, e.g., cellular recognition, immunity, embryonic development, carcinogenesis and cell-death. Thus, whereas two naturally occurring amino acids can be used by nature to

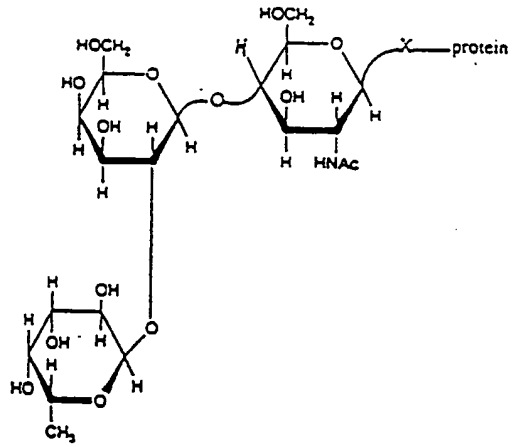
convey 2 fundamental molecular messages, i.e., via formation of the two possible dipeptide structures, and four different nucleotides convey 24 molecular messages, two different monosaccharide subunits can give rise to 11 unique disaccharides, and four dissimilar monosaccharides can give rise to up to 35,560 unique tetramers, each capable of functioning as a fundamental discreet molecular messenger in a given physiological system.

The gangliosides are examples of the versatility and effect with which organisms can use saccharide structures. These molecules are glycolipids (sugar-lipid composites) and as such are able to position themselves at strategic locations on the cell wall: their lipid component enables them to anchor in the hydrophobic interior of the cell wall, positioning their hydrophilic component in the aqueous extracellular milieu. Thus the gangliosides (like many other saccharides) have been chosen to act as cellular sentries: they are involved in both the inactivation of bacterial toxins and in contact inhibition, the latter being the complex and poorly understood process by which normal cells inhibit the growth of adjacent cells, a property lost in most tumor cells. The structure of ganglioside GM, a potent inhibitor of the toxin secreted by the cholera organism, featuring a branched complex pentameric structure is shown below.

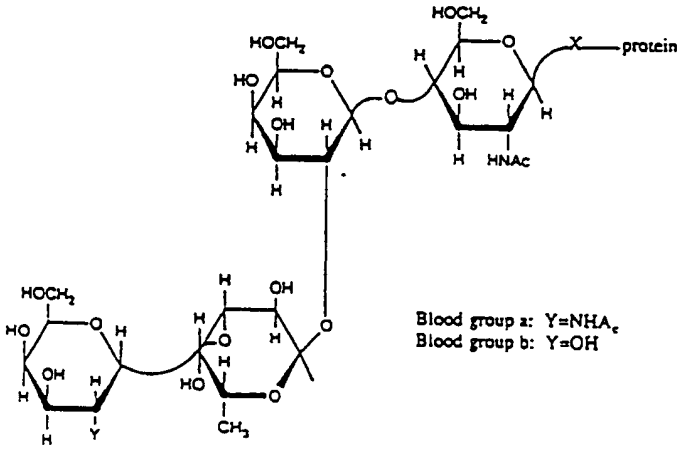


GANGLIOSIDE GM_1

The oligosaccharide components of the glycoproteins (sugar-protein composites) responsible for the human blood-group antigens (the A, B, and O blood classes) are shown below:



BLOOD GROUP O ANTIGEN, TYPE II



BLOOD GROUP A AND B ANTIGENS

Interactions involving complementary proteins and glycoproteins on red blood cells belonging to incompatible blood classes cause formation of aggregates, or clusters and are the cause for failed transfusions of human blood.

Numerous other biological processes and macromolecules are controlled by glycosylation (i.e., the covalent linking with sugars). Thus, deglycosylation of erythropoetin causes loss of the hormone's biological activity; deglycosylation of human gonadotropic hormone increases receptor binding but results in almost complete loss of biological activity (see Rademacher et al., Ann. Rev. Biochem 57, 785 (1988); and glycosylation of three sites in tissue plasminogen activating factor (TPA) produces a glycopolypeptide which is 30% more active than the polypeptide that has been glycosylated at two of the sites.

2.1.3 Design and Synthesis of Mimetics of Biological Ligands

A currently favored strategy for the development of agents which can be used to treat diseases involves the discovery of forms of ligands of biological receptors, enzymes, or related macromolecules, which mimic such ligands and either boost, i.e., agonize, or suppress, i.e., antagonize, the activity of the ligand. The discovery of such desirable ligand forms has traditionally been carried out either by random screening of molecules (produced through chemical synthesis or isolated from natural sources), or by using a so-called "rational" approach involving identification of a lead-structure, usually the structure of the native ligand, and optimization of its properties through numerous cycles of structural redesign and biological testing. Since most useful drugs have been

discovered not through the "rational" approach but through the screening of randomly chosen compounds, a hybrid approach to drug discovery has recently emerged which is based on the use of combinatorial chemistry to construct huge libraries of randomly-built chemical structures which are screened for specific biological activities. (S. Brenner and R.A. Lerner,. 1992, Proc. Natl. Acad. Sci. USA 89:5381)

Most lead-structures which have been used in "rational" drug design are native polypeptide ligands of receptors or enzymes. The majority of polypeptide ligands, especially the small ones, are relatively unstable in physiological fluids, due to the tendency of the peptide bond to undergo facile hydrolysis in acidic media or in the presence of peptidases. Thus, such ligands are decisively inferior in a pharmacokinetic sense to nonpeptidic compounds, and are not favored as drugs. An additional limitation of small peptides as drugs is their low affinity for ligand acceptors. This phenomenon is in sharp contrast to the affinity demonstrated by large, folded polypeptides, e.g., proteins, for specific acceptors, e.g., receptors or enzymes, which can be in the subnanomolar range. For peptides to become effective drugs, they must be transformed into nonpeptidic organic structures, i.e., peptide mimetics, which bind tightly, preferably in the nanomolar range, and can withstand the chemical and biochemical rigors of coexistence with biological tissues and fluids.

Despite numerous incremental advances in the art of peptidomimetic design, no general solution to the problem of converting a polypeptide-ligand structure to a peptidomimetic has been defined. At present, "rational" peptidomimetic design is done on an ad hoc basis. Using numerous redesign-synthesis-screening cycles, peptidic ligands belonging to a certain biochemical class have been converted by groups of organic chemists and pharmacologists to specific peptidomimetics: however, in the majority of cases the results in one biochemical area, e.g., peptidase inhibitor design using

the enzyme substrate as a lead, cannot be transferred for use in another area, e.g., tyrosine-kinase inhibitor design using the kinase substrate as a lead.

In many cases, the peptidomimetics that result from a peptide structural lead using the "rational" approach comprise unnatural alpha-amino acids. Many of these mimetics exhibit several of the troublesome features of native peptides (which also comprise alpha-amino acids) and are, thus, not favored for use as drugs. Recently, fundamental research on the use of nonpeptidic scaffolds, such as steroidal or sugar structures, to anchor specific receptor-binding groups in fixed geometric relationships have been described (see for example Hirschmann, R. et al., 1992 J. Am. Chem. Soc., 114:9699-9701; Hirschmann, R. et al., 1992 J. Am. Chem. Soc., 114:9217-9218); however, the success of this approach remains to be seen.

In an attempt to accelerate the identification of lead-structures, and also the identification of useful drug candidates through screening of randomly chosen compounds, researchers have developed automated methods for the generation of large combinatorial libraries of peptides and certain types of peptide mimetics, called "peptoids", which are screened for a desirable biological activity. For example, the method of H. M. Geysen, (1984 Proc. Natl. Acad. Sci. USA 81:3998) employs a modification of Merrifield peptide synthesis, wherein the C-terminal amino acid residues of the peptides to be synthesized are linked to solid-support particles shaped as polyethylene pins; these pins are treated individually or collectively in sequence to introduce additional amino-acid residues forming the desired peptides. The peptides are then screened for activity without removing them from the pins. Houghton, (1985, Proc. Natl. Acad. Sci. USA 82:5131; and U.S. Patent No. 4,631,211) utilizes individual polyethylene bags ("tea bags") containing C-terminal amino acids bound to a solid support. These are mixed and coupled with the requisite amino acids using solid phase synthesis

techniques. The peptides produced are then recovered and tested individually. Fodor et al., (1991, Science 251:767) described light-directed, spatially addressable parallel-peptide synthesis on a silicon wafer to generate large arrays of addressable peptides that can be directly tested for binding to biological targets. These workers have also developed recombinant DNA/genetic engineering methods for expressing huge peptide libraries on the surface of phages (Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA 87:6378).

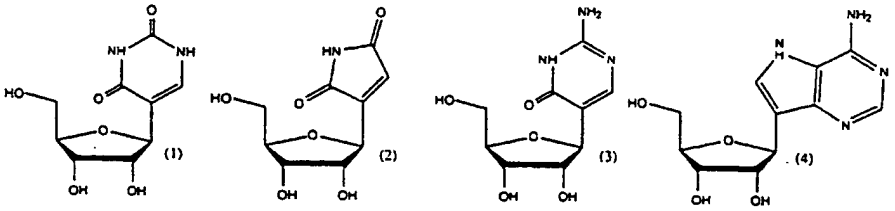
In another combinatorial approach, V. D. Huebner and D.V. Santi (U.S. Patent No. 5,182,366) utilized functionalized polystyrene beads divided into portions each of which was acylated with a desired amino acid; the bead portions were mixed together, then divided into portions each of which was re-subjected to acylation with a second desirable amino acid producing dipeptides, using the techniques of solid phase peptide synthesis. By using this synthetic scheme, exponentially increasing numbers of peptides were produced in uniform amounts which were then separately screened for a biological activity of interest.

Zuckerman et al., (1992, Int. J. Peptide Protein Res. 91:1) also have developed similar methods for the synthesis of peptide libraries and applied these methods to the automation of a modular synthetic chemistry for the production of libraries of N-alkyl glycine peptide derivatives, called "peptoids", which are screened for activity against a variety of biochemical targets. (See also, Symon et al., 1992, Proc. Natl. Acad. Sci. USA 89:9367). Encoded combinatorial chemical syntheses have been described recently (S. Brenner and R.A. Lerner, 1992, Proc. Natl. Acad. Sci. USA 89:5381).

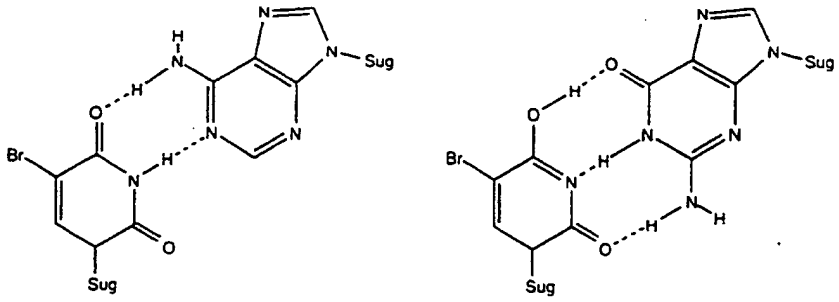
Recently in an alternate strategy for the design of therapeutically active mimetic ligands much attention has been focused on the construction and application of molecules which possess the property of binding to nucleic acids. These materials, whether they be direct Watson-Crick type "antisense" nucleotide mimetics, Hoogstein-type binders or

minor groove binding compounds such as those pioneered by Dervan and coworkers, have employed a variety of derivatives and variants of the naturally occuring sugar-phosphate backbone. Polyamide backbones have also been employed to support the base complements. While binding and desired functionality is observed *in vitro* withthese systems, they have inherent design drawbacks for *in vivo* use for hybridization against a rogue gene or its insidious RNA. The two main drawbacks of these polyamide systems are in (a) the persistent reliance upon an amide bond which is susceptible to proteolytic cleavage, and (b) the inability of the compound either as a class, or even singularly show efficient membrane permeability.

However, in the course of this work, a great amount of knowledge has been amassed *vis-a-vis* 1.) the ability of a synthetic scaffold to support a series of natural or designed bases in such a manner that tight binding to natural nucleic acids is observed; 2.) the requirements for designed or naturally occurring nucleotide bases other than guanosine, cytosine, thymidine, adenine, or uridine, to efficiently hydrogen bond (hybridize) to another, natural base or nucleotide. Among these natural nucleotide mimetics are showdomycin (1) and pseudouridine (2) and the synthetic compounds (3) and (4).



It has been demonstrated that such unnatural or modified bases can show efficient hybridization if projected from an effective scaffold as shown here for both tautomers of 5-bromouracil, which can bind to either adenine or guanine



The primary goal of any "antisense" or "gene therapy" is to inactivate the archival rogue information (deliterious DNA) or the messenger information (the correpsonding RNA) by very tight, specific hybridization.

As previously stated, there are a multitude of paths by which the "anti-sense" agent may be metabolized or destroyed outright, and as a result of these known obstacles, chemists have pursued alternative backbones that might enable their compounds to (a) survive the degradative response of the immune and metabolic pathways, and (b) transit the cellular and nuclear membranes to the site at which hybridization may occur.

In addition to the lead structure, a very useful source of information for the realization of the preferred "rational" drug discovery is the structure of the biological ligand acceptor which, often in conjunction with molecular modelling calculations, is used to simulate modes of binding of the ligand with its acceptor; information on the mode of binding is useful in optimizing the binding properties of the lead-structure. However, finding the structure of the ligand acceptor, or preferably the structure of a complex of the acceptor with a high affinity ligand, requires the isolation of the acceptor or complex in the pure, crystalline state, followed by x-ray crystallographic analysis. The isolation and purification of biological receptors, enzymes, and the polypeptide substrates thereof are time-consuming, laborious, and expensive. Success in this important area of biological

chemistry depends on the effective utilization of sophisticated separation technologies.

Crystallization can be valuable as a separation technique but in the majority of cases, especially in cases involving isolation of a biomolecule from a complex biological milieu, successful separation is chromatographic. Chromatographic separations are the result of reversible differential binding of the components of a mixture as the mixture moves on an active natural, synthetic, or semisynthetic surface; tight-binding components in the moving mixture leave the surface last en masse resulting in separation.

The development of substrates or supports to be used in separations has involved either the polymerization-crosslinking of monomeric molecules under various conditions to produce fabricated materials such as beads, gels, or films, or the chemical modification of various commercially available fabricated materials e.g., sulfonation of polystyrene beads, to produce the desired new materials. In the majority of cases, prior art support materials have been developed to perform specific separations or types of separations and are thus of limited utility. Many of these materials are incompatible with biological macromolecules, e.g., reverse-phase silica frequently used to perform high pressure liquid chromatography can denature hydrophobic proteins and other polypeptides. Furthermore, many supports are used under conditions which are not compatible with sensitive biomolecules, such as proteins, enzymes, glycoproteins, etc., which are readily denaturable and sensitive to extreme pH's. An additional difficulty with separations carried out using these supports is that the separation results are often support-batch dependent, i.e. they are irreproducible.

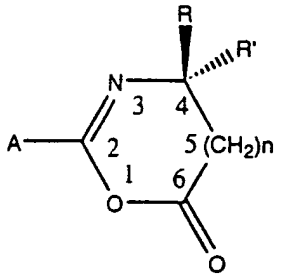
Recently a variety of coatings and composite-forming materials have been used to modify commercially available fabricated materials into articles with improved properties; however the success of this approach remains to be seen.

If a chromatographic support is equipped with molecules which bind specifically with a component of a complex mixture, that component will be separated from the mixture and may be released subsequently by changing the experimental conditions (e.g. , buffers, stringency, etc.) This type of separation is appropriately called "affinity chromatography" and remains an extremely effective and widely used separation technique. It is certainly much more selective than traditional chromatographic techniques, e.g chromatography on silica, alumina, silica or alumina coated with long-chain hydrocarbons, polysaccharide and other types of beads or gels which in order to attain their maximum separating efficiency need to be used under conditions that are damaging to biomolecules, e.g., conditions involving high pressure, use of organic solvents and other denaturing agents, etc.

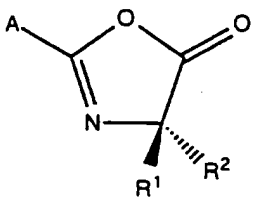
The development of more powerful separation technologies depends significantly on breakthroughs in the field of materials science, specifically in the design and construction of materials that have the power to recognize specific molecular shapes under experimental conditions resembling those found in physiological media, i.e. , these experimental conditions must involve an aqueous medium whose temperature and pH are close to the physiological levels and which contains none of the agents known to damage or denature biomolecules. The construction of these "intelligent" materials frequently involves the introduction of small molecules capable of specifically recognizing others into existing materials, e.g. surfaces, films, gels, beads, etc., by a wide variety of chemical modifications; alternatively molecules capable of recognition are converted to monomers and used to create the "intelligent" materials through polymerization reactions.

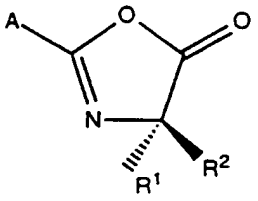
2.2 Oxazolones

Oxazolones, or azlactones, are structures of the general formula:



where A is a functional group and n is 0-3. Oxazolones containing a five-membered ring and a single substituent at position 4 are typically encountered as transient intermediates which cause problematic racemization during the chemical synthesis of peptides. An oxazolone can in principle contain one or two substituents at the 4-position. When these substituents are not equivalent, the carbon atom at the 4-position is asymmetric and two non-superimposable oxazolone structures (azlactones) result:

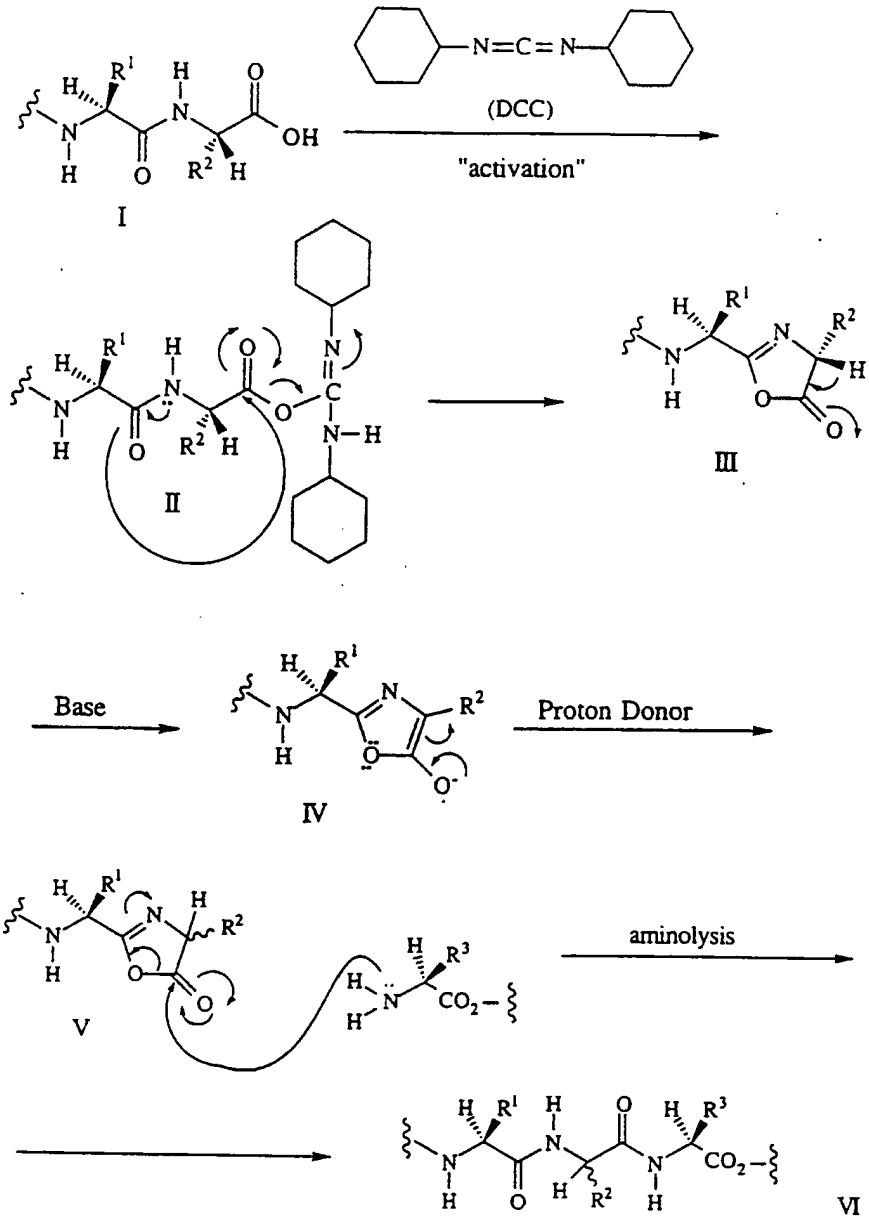




Chiral oxazolones possessing a single 4 substituent (also known as 5(4H)-oxazolones), derived from (chiral) natural amino acid derivatives, including activated acylamino acyl structures, have been prepared and isolated

acylamino acyl structures, have been prepared and isolated in the pure, crystalline state (Bodansky, M.; Klausner, Y. S.; Ondetti, M. A. in "Peptide Synthesis", Second Edition, John Wiley & Sons, New York, 1976, p. 14 and references cited therein). The facile, base-catalyzed racemization of several of these oxazolones has been studied in connection with investigations of the serious racemization problem confronting peptide synthesis (see Kemp, D. S. in "The Peptides, Analysis, Synthesis, and Biology", Vol. 1, Gross, E. & Meienhofer, J. editors, 1979, p. 315).

Racemization during peptide synthesis becomes very extensive when the desired peptide is produced by aminolysis of activated peptidyl carboxyl, as in the case of peptide chain extension from the amino terminus, e.g. I _ VI shown below (see Atherton, E.; Sheppard, R. C. "Solid Phase Peptide Synthesis, A Practical Approach", IRL Press at Oxford University Press, 1989, pages 11 and 12). An extensively studied mechanism describing this racemization involves conversion of the activated acyl derivative (II) to an oxazolone (III) followed by facile base-catalyzed racemization of the oxazolone via a resonance-stabilized intermediate (IV) and aminolysis of the racemic oxazolone (V) producing racemic peptide products (VI).

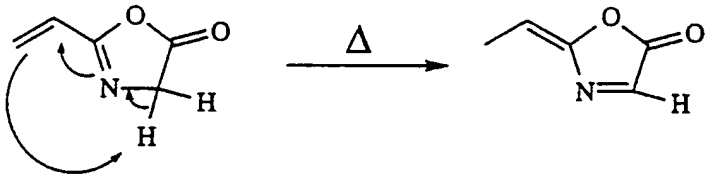


Extensive research on the trapping of oxazolones III (or of their activated acyl precursors II) to give acylating agents which undergo little or no racemization upon aminolysis has been carried out, and successes in this

area (such as the use of N-hydroxybenzotriazole) have greatly advanced the art of peptide synthesis (Kemp, D. S. in "The Peptides, Analysis, Synthesis, and Biology", Vol. 1, Gross, E. & Meienhofer, J. editors, 1979, p. 315).

Thus, attempts to deal with the racemization problem in peptide synthesis have involved suppressing or avoiding the formation of oxazolone intermediates altogether.

Furthermore, certain vinyl oxazolones having a hydrogen substituent at the 4-position can also undergo thermal rearrangements (23 Tetrahedron 3363 (1967)), which may interfere with other desired transformations, such as Michael-type additions.



3. SUMMARY OF THE INVENTION

A new approach to the construction of novel molecules is described. This approach involves the development of oxazolone (azlactone) derived molecular building blocks, containing appropriate atoms and functional groups, which may be chiral and which are used in a modular assembly of molecules with tailored properties; each module contributing to the overall properties of the assembled molecule. The oxazolone-derivative building blocks of the invention can be used to synthesize novel molecules designed to mimic the three-dimensional structure and function of native ligands.

and/or interact with the binding sites of a native receptor. This logical approach to molecular construction is applicable to the synthesis of all types of molecules, including but not limited to mimetics of peptides, proteins, oligonucleotides, carbohydrates, lipids, polymers and to fabricated materials useful in materials science. It is analogous to the modular construction of a mechanical apparatus that performs a specific operation wherein each module performs a specific task contributing to the overall operation of the apparatus.

The invention is based, in part, on the following insights of the discoverer. (1) All ligands share a single universal architectural feature: they consist of a scaffold structure, made e.g. of amide, carbon-carbon, or phosphodiester bonds which support several functional groups in a precise and relatively rigid geometric arrangement. (2) Binding modes between ligands and receptors share a single universal feature as well: they all involve attractive interactions between complementary structural elements, e.g., charge- and pi-type interactions, hydrophobic and van der Waals forces, hydrogen bonds. (3) A continuum of fabricated materials exists spanning a dimensional range from about 100 Angstroms to 1 cm in diameter comprising various materials of construction, geometries, morphologies, and functions, all possessing the common feature of a functional surface which is presented to a biologically active molecule or a mixture of molecules to achieve recognition between the molecule (or the desired molecule in a mixture) and the surface. And (4) Oxazolone derivative structures, heretofore regarded as unwanted intermediates which may form during the synthesis of peptides, would be ideal building blocks for constructing backbones or scaffolds bearing the appropriate functional groups that either mimic desired ligands, and/or interact with appropriate receptor binding sites, and for carrying out the synthesis of the various parts of the functionalized scaffold orthogonally, provided that racemization of the

oxazolone structures is prevented or controlled. Thus, the invention is also based, in part, on the further recognition that such derivatives of ozaxolones, which do not racemize, can be used as universal building blocks for the synthesis of such novel molecules. Furthermore, oxazolone derivatives may be utilized in a variety of ways across the continuum of fabricated materials described above to produce new materials capable of specific molecular recognition. These oxazolone derivatives may be chirally pure and used to synthesize molecules that mimic a number of biologically active molecules, including but not limited to peptides, proteins, oligonucleotides, polynucleotides, carbohydrates and lipids, and a variety of other polymers as well as fabricated materials that are useful as new materials, including but not limited to solid supports useful in column chromatography, catalysts, solid phase immunoassays, drug delivery vehicles, films, and "intelligent" materials designed for use in selective separations of various components of complex mixtures.

Working examples describing the use of oxazolone derived modules in the modular assembly of a variety of molecular structures are given. The molecular structures include functionalized silica surfaces useful in the optical resolution of racemic mixtures; peptide mimetics which inhibit human elastase, protein-kinase, and the HIV protease; carbohydrate, oligonucleotide and pharmacophore mimetics and polymers formed via free-radical or condensation polymerization of oxazolone-containing monomers.

In accordance with the present invention, the oxazolone-derived molecules of interest possess the desired stereochemistry and, when required, are obtained enantiomerically pure. In addition to the synthesis of single molecular entities, the synthesis of libraries of oxazolone-derived molecules, using the techniques described herein or modifications thereof which are well known in the art to

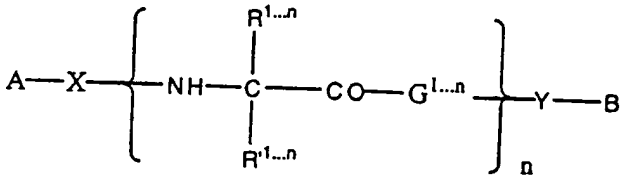
perform combinatorial chemistry, is also within the scope of the invention. Furthermore, the oxazolone-derived molecules possess enhanced hydrolytic and enzymatic stabilities, and in the case of biologically active materials, are transported to target ligand-acceptor macromolecules *in vivo*, without causing any serious side-effects.

According to the present invention, chiral oxazolones, in which the asymmetric center is a disubstituted carbon at the 4-position, as well as synthetic nonchiral oxazolones may be synthesized readily and used as molecular modules capable of controlled reaction with a variety of other molecules to produce designed chiral recognition agents and conjugates. These chiral oxazolones may also be linked together, using polymerizing reactions carried out either in a stepwise or chain manner, to produce polymeric biological ligand mimics of defined sequence and stereochemistry. Furthermore, according to the present invention, 4-disubstituted chiral oxazolones are extremely useful in the asymmetric functionalization of various solid supports and biological macromolecules and in the production of various chiral polymers with useful properties. The products of all of these reactions are surprisingly stable in diverse chemical and enzymological environments, and uniquely suitable for a variety of superior pharmaceutical and high-technological applications.

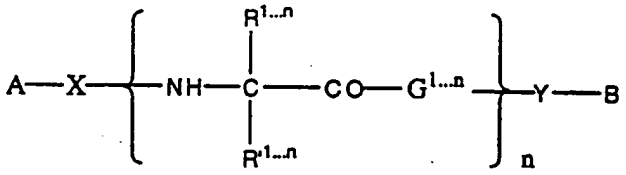
For applications in which the 4 position of the oxazolone precursor does not need to be chiral, e.g., the construction of certain polymeric materials, the use of oxazolones in the construction of linkers for the joining of two or more pharmaceutically useful or, simply, biologically active ligands, etc., symmetric or nonchiral oxazolones are used in chemical syntheses. Furthermore, if the oxazolone-derived product does not need to incorporate the 4-position of the oxazolone precursor in the enantiomerically pure

state, oxazolone precursors which are not enantiomerically pure may be used for syntheses.

The invention is also directed to a method of making a polymer having a particular water solubility comprising the steps of; a) choosing a first monomer having the formula



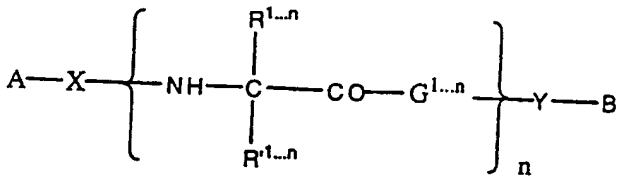
wherein R and R' are the same or different and are chosen from those organic moieties exhibiting hydrophobicity; b) choosing a second monomer having the formula



wherein R and R' are the same or different and are chosen from those organic moieties exhibiting hydrophilicity; and c) reacting said monomers to provide an effective amount of each monomer in a developing polymer chain until a polymer having the desired water solubility is created. According to this method said hydrophobic organic moieties can include those which do not have carboxyl, amino or ester functionality. Also said hydrophilic moieties can include those which do have carboxyl, amino or ester functionality.

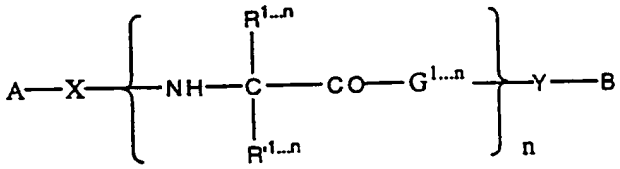
This invention is further directed to using said method of preparing a synthetic compound to produce a compound that mimics or complements the structure of a biologically active compound of the formula. This method can be used to produce pharmacaphores, peptide mimetics, nucleotide mimetics, carbohydrate mimetics, and reporter compounds, for example.

This invention is also further directed to a method of preparing a combinatorial library which comprises: a) preparing a compound having the formula;



$n \geq 1$; and b) conducting further reactions with the compound to form a combinatorial library.

Still further this invention is directed to a method of separating a desired compound from a plurality of compounds, which comprises; a) preparing a separator compound having the formula:



$n \geq 1$;

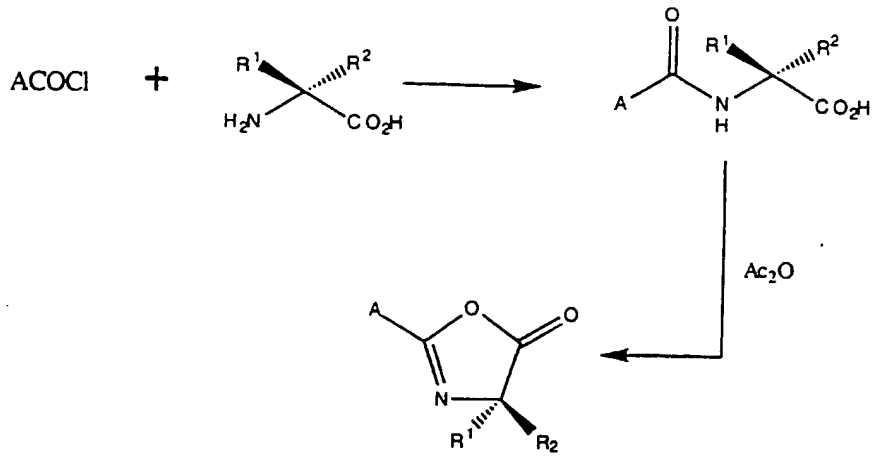
b) contacting said separator compound with the plurality of compounds; and c) differentiating said second compound and the separated compounded from said plurality of compounds.

different ways. The compounds of the present invention can be synthesized by many different routes. It is well known in the art of organic synthesis that many different synthetic protocols can be used to prepare a given compound. Different routes can involve more or less expensive reagents, easier or more difficult separation or purification procedures, straightforward or cumbersome scale up, and higher or lower yield. The skilled synthetic organic chemist knows well how to balance the competing characteristics of synthetic strategies. Thus the compounds of the present invention are not limited by the choice of synthetic strategy, and any synthetic strategy that yields the compounds described below can be used.

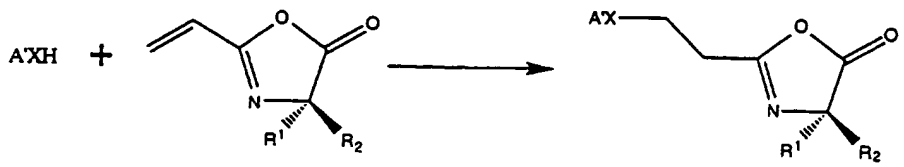
4.1 Synthesis of Chiral Substituted Oxazolones

Chiral 4,4'-disubstituted oxazolones may be prepared from the appropriate N-acyl amino acid using any of a number of standard acylation and cyclization techniques well-known to those skilled in the art, e.g.:

The scope of this invention is intended to encompass each species of the aforementioned Markush genus. Thus, for example, where there is a numeric designation in the claim, that can be an integer, i.e. m or n, the scope of this invention is intended to cover each species that would be represented by every different integer.

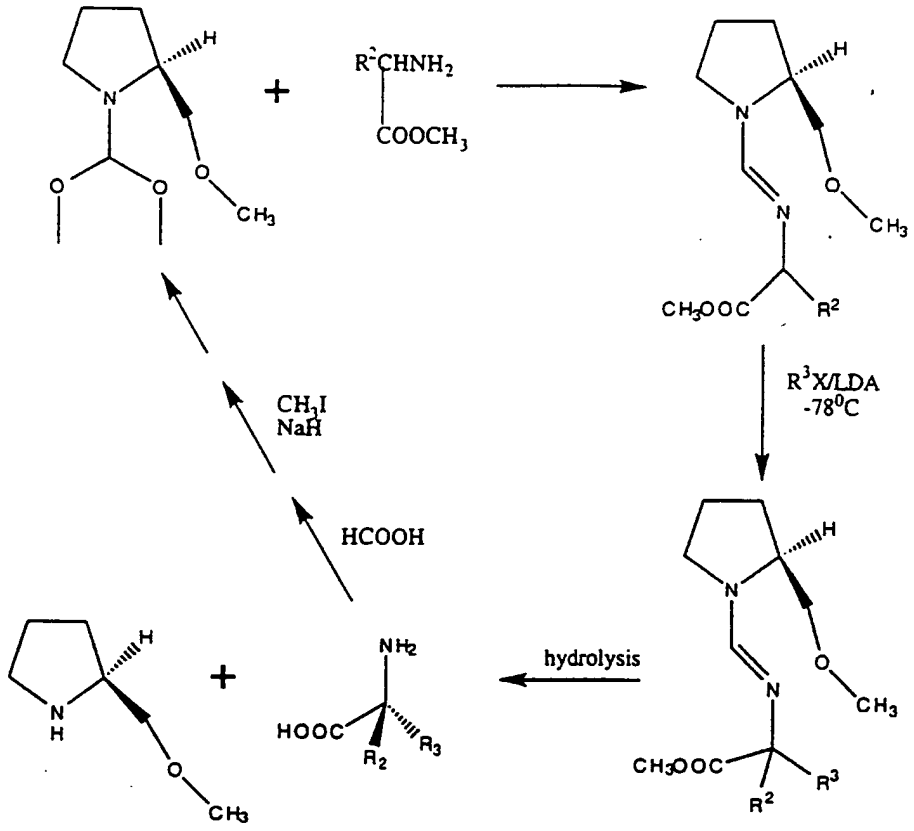


When the substituent at the 2-position is capable of undergoing addition reactions, these may be carried out with retention of the chirality at the 4-position to produce new oxazolones. This is shown for the Michael-type addition to an alkenyl oxazolone as follows:

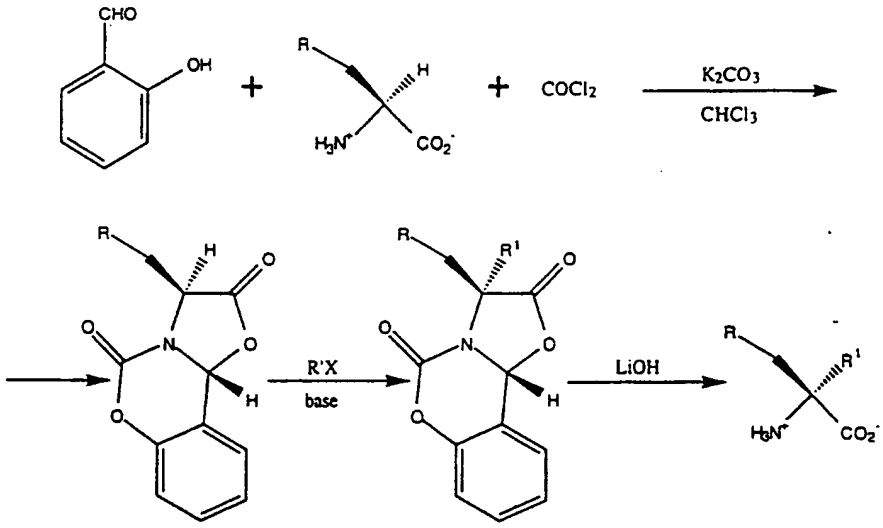


where X = S or NR and A' is a functional group.

The required chiral amino acid precursors for oxazolone synthesis may be produced using stereoselective reactions that employ chiral auxiliaries. An example of such a chiral auxiliary is (5)-(-)-1-dimethoxymethyl-2-methoxymethylpyrrolidine (SMPD) (Liebig's Ann. Chem. 1668 (1983)) as shown below,

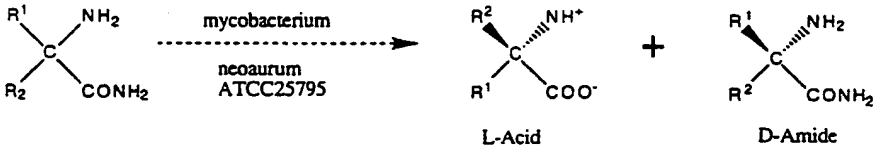


wherein R^2 = CH_3, *i*-Bu, or benzyl; and R^3 = CH_3, CHF_2, C_2H_5, *n*-Bu, or benzyl. A second example involves 5*H*,10ß-Hoxazolo[3,2-c][1,3]benzoxazine-2(3H),5-diones (55 J. Org. Chem. 5437 (1990)),



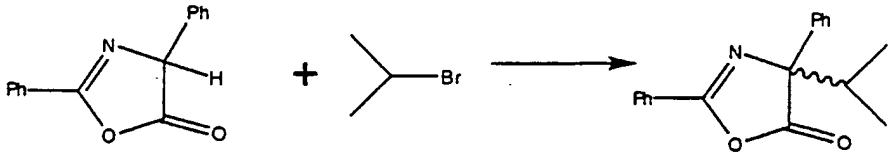
wherein R^1 = phenyl or i-Pr; and R^2 = CH_3, C_2H_5, or CH_2=CH-CH_2.

Alternatively, the desired chiral amino acid may be obtained using stereoselective biochemical transformations carried out on the racemate, synthesized via standard reactions, as shown below for a case involving a commercially-available organism (53 J. Org. Chem. 1826 (1988)),



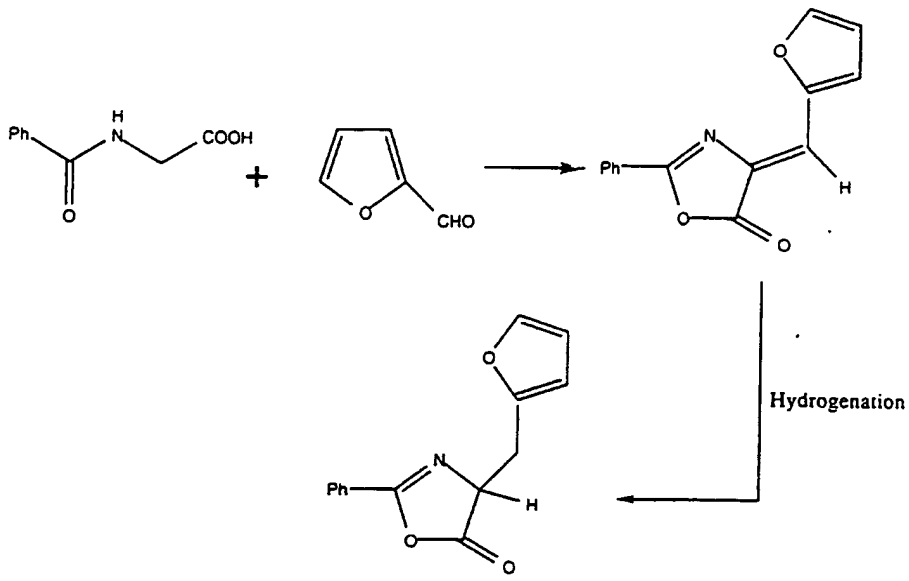
wherein R^1 = i-Pr, i-Bu, phenyl, benzyl, p-methoxybenzyl, or phenethyl; and R = CH_3 or C_2H_5.

Racemic mixtures of 4,4'-disubstituted oxazolones may be prepared from monosubstituted oxazolones by alkylation of the 4-position, as in the following transformation (Synthesis Commun., Sept. 1984, at 763; 23 Tetrahedron Lett. 4259 (1982)):



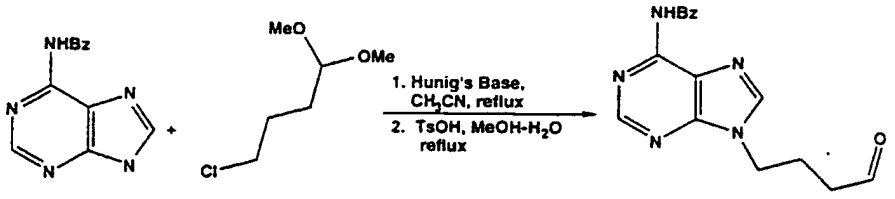
Resolution of racemic mixtures of oxazolones may be effected using chromatography or chiral supports under suitable conditions which are well known in the art; using fractional crystallization of stable salts of oxazolones with chiral acids; or simply by hydrolyzing the racemic oxazolone to the amino acid derivative and resolving the racemic modification using standard analytical techniques.

A wide variety of 4-monosubstituted azlactones may be readily prepared by reduction of the corresponding unsaturated derivatives obtained in high yield from the condensation reaction of aldehydes, ketones, or imines with the oxazolone formed from an N-acyl glycine (49 J. Org. Chem. 2502 (1984); 418 Synthesis Communications (1984))

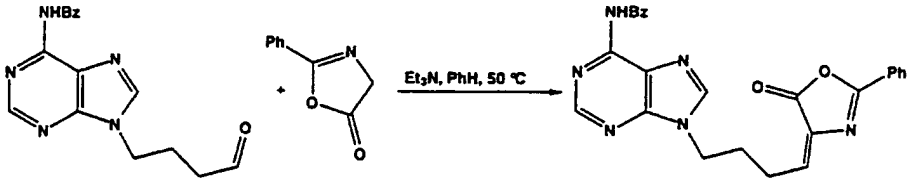


The hydrogenation may be carried out using a stereospecific hydrogenation catalyst to produce an enantiomerically pure product. This product may subsequently be stereoselectively alkylated to produce the enantiomeric disubstituted oxazolone module. An example of this is given below for the synthesis of an enantiomerically pure adenine-derivatized nucleotide mimetic oxazolone module:

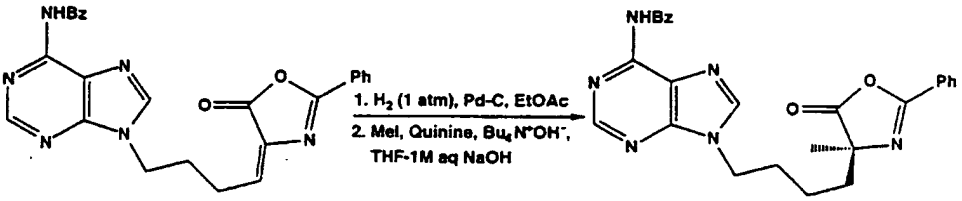
Step 1. - Attachment of a Carbonyl-terminal Spacer



Step 2. - Coupling to the Oxazolone 4-Position



Step 3. - Stereospecific Reduction and Phase Transfer Methylation

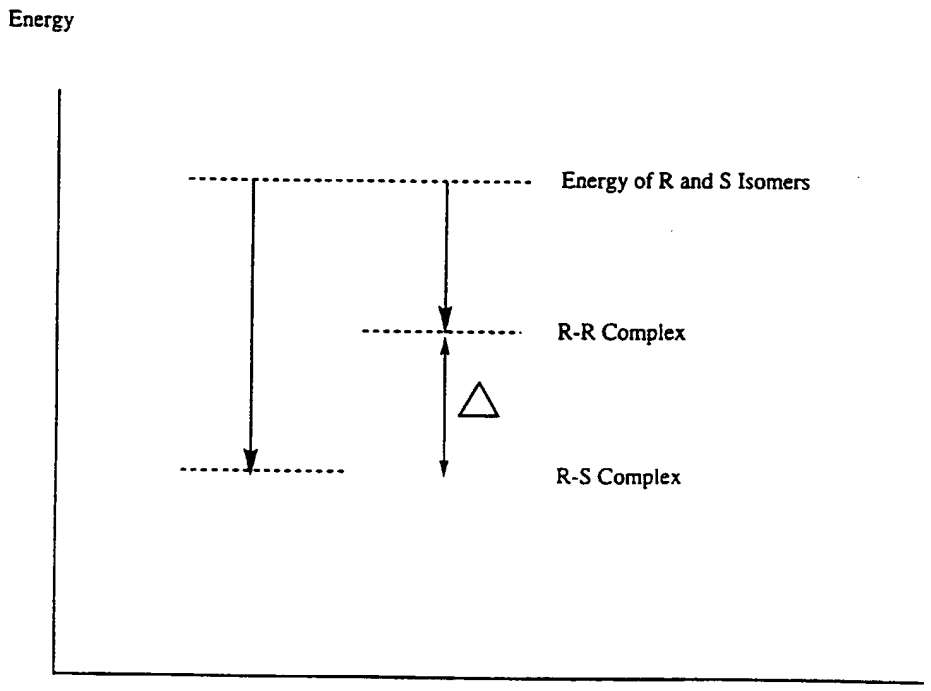


Thus, there are numerous chemical and biochemical methods which can be used to produce a wide variety of enantiomeric, multifunctionalized oxazolones whose substituents may be tailored to mimic any desirable form of the side chains of native polypeptides and oligonucleotides, mimics and variants of these, carbohydrate and pharmacophore variants and mimetics, or any other side chain substituent which can be attached to a scaffold or a backbone to produce a desired interaction with a target system.

4.2 Chiral Recognition

"Chiral recognition" is a process whereby individual chiral enantiomers display differential binding energies with an enantiomerically pure chiral target or recognition agent. This agent may be attached to a surface to produce a chiral stationary phase (CSP) for chromatographic use or may be used to form diastereomeric complexes with the racemic target. These complexes have differing physiochemical propereties which allow them to be separated using standard unit processes, such as fractional crystallization.

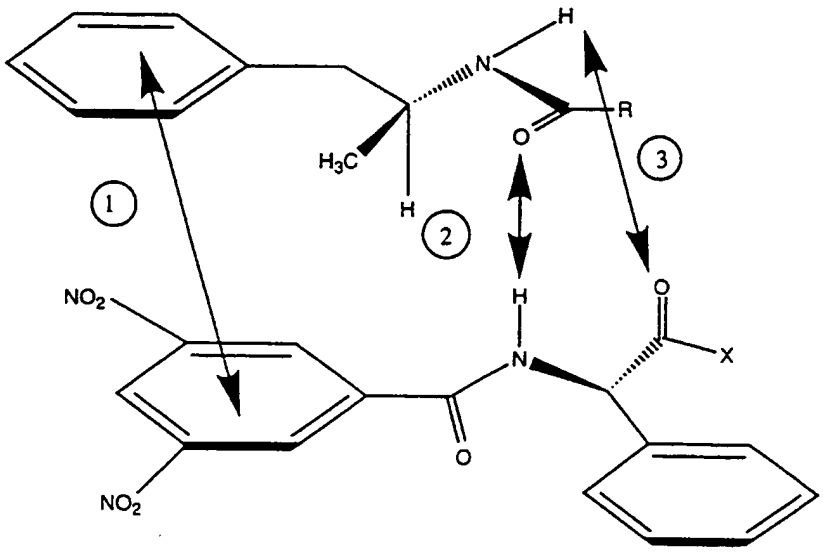
Two steps are necessary for this recognition process to occur with a CSP; 1.) absorption and 2.) energetic differentiation between the enantiomers. The absolute binding energies between the enantiomers and the surface determine the tightness of the binding. The difference in energy between the complexes determines the selectivity. This is represented in the following diagram



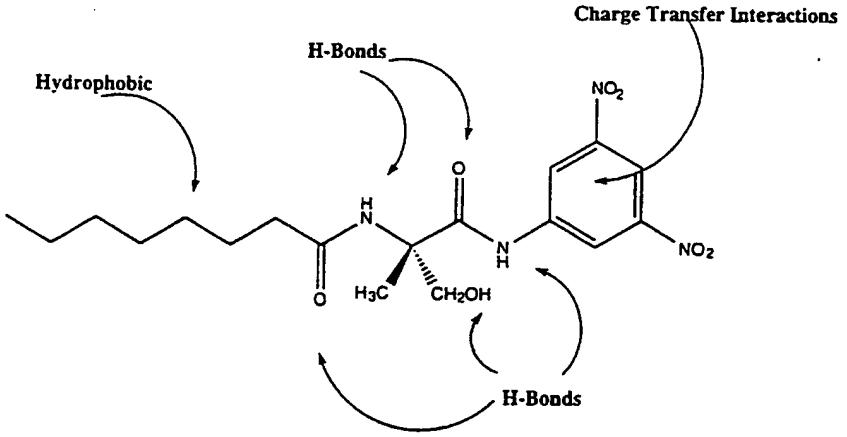
The interaction of the enantiomeric R and S species with the CSP can be envisioned as a "three point interaction". This does not mean that three actual points of attachment or association are necessary, but rather that any three kinds of attractive or repulsive interactions within the diastereomeric complexes can serve to differentiate ("recognize") the enantiomers. Greater differentiation ("recognition") betwen the complexes is promoted by multiple combinations of attractive and/or repulsive interactions, including hydrogen bonding, ionic interactions, dipole interactions, hydrophobic, pi-pi interactions and steric interactions between the two chiral species. The larger the number and the more varied the types of these interactions, the greater the resulting energy differences between the complexex and the greater the degree of "recognition" per interaction.

This is figuratively illustrated below:

"Three point interaction"



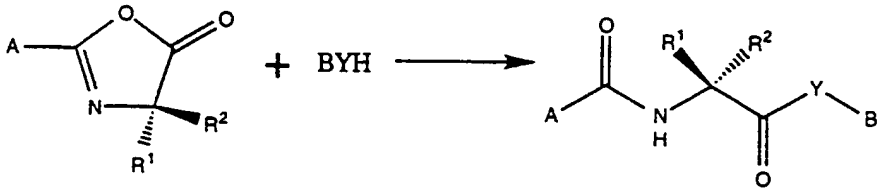
The possible modes of interaction which can participate in such "three point interactions" is depicted below for a enantiomerically pure oxazolone derivative:



4.3 Synthetic Transformations of Chiral Oxazolones

4.3.1 Reactions with One or Two Nucleophiles Producing Conjugates

Chiral oxazolones may be subjected to ring opening reactions with a variety of nucleophiles producing chiral molecules as shown below:



In the structure above, Y represents an oxygen, sulfur, or nitrogen atom. R^1 and R^2 differ from one another and taken alone each signifies one of the following: alkyl

including carbocyclic and substituted forms thereof; aryl, aralkyl, alkaryl, and substituted or heterocyclic versions thereof; preferred forms of R1 and R2 are the side chain substituents occurring in native polypeptides, oligonucleotides, variants or mimetics of these, carbohydrates, pharmacophores, variants or mimetics of these, or any other side chain substituent which can be attached to a scaffold or a backbone to produce a desired interaction with a target system.

The above ring-opening reaction can be carried out either in an organic solvent such as methylene chloride, ethyl acetate, dimethyl formamide (DMF) or in water at room or higher temperatures, in the presence or absence of acids, such as carboxylic, other proton or Lewis-acids, or bases, such as tertiary amines or hydroxides, serving as catalysts. If structure BYH contains nucleophilic functional groups which may interfere with the ring-opening acylation, these groups must be temporarily protected using suitable orthogonal protection strategies based on the many protecting groups known in the art; cf., e.g., Protective Groups in Organic Synthesis, 2ed., T. W. Greene and P. G. M. Wuts, John Wiley & Sons, New York, N.Y., 1991.

The substituents A and B shown may be of a variety of structures and may differ markedly in their physical or functional properties, or may be the same; they may also be chiral or symmetric. A and B are preferably selected from:

1) Amino acid derivatives of the form (AA)N, which would include, for example, natural and synthetic amino acid residues (N=1) including all of the naturally occuring alpha amino acids, especially alanine, arginine, asparagnine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine; the naturally occuring disubstituted amino acids, such as amino isobutyric acid, and isovaline, etc.; a variety of synthetic amino acid residues,

including alpha-disubstituted variants, species with olefinic substitution at the alpha position, species having derivatives, variants or mimetics of the naturally occuring side chains; N-Substituted glycine residues; natural and synthetic species known to functionally mimic amino acid residues, such as statine, bestatin, etc. Peptides (N=2-30) constructed from the amino acids listed above, such as angiotensinogen and its family of physiologically important angiotensin hydrolysis products, as well as derivatives, variants and mimetics made from various combinations and permutations of all the natural and synthetic residues listed above. Polypeptides (N=31-70), such as big endothelin, pancreastatin, human growth hormone releasing factor and human pancreatic polypeptide.

Proteins (N>70) including structural proteins such as collagen, functional proteins such as hemoglobin, regulatory proteins such as the dopamine and thrombin receptors.

2) Nucleotide derivatives of the form (NUCL)N, which includes natural and synthetic nucleotides (N=1) such as adenosine, thymine, guanidine, uridine, cystosine, derivatives of these and a variety of variants and mimetics of the purine ring, the sugar ring, the phosphate linkage and combinations of some or all of these. Nucleotide probes (N=2-25) and oligonucleotides (N>25) including all of the various possible homo and heterosynthetic combinations and permutations of the naturally occuring nucleotides, derivatives and variants containing synthetic purine or pyrimidine species or mimics of these, various sugar ring mimetics, and a wide variety of alternate backbone analogues including but not limited to phosphodiester, phosphorothionate, phosphorodithionate, phosphoramidate, alkyl phosphotriester, sulfamate, 3'-thioformacetal, methylene(methylimino), 3-N-carbamate, morpholino carbamate and peptide nucleic acid analogues.

3) Carbohydrate derivatives of the form (CH)n. This would include natural physiologically active carbohydrates such as including related compounds such as glucose, galactose, sialic acids, beta-D-glucosylamine and nojorimycin which are

both inhibitors of glucosidase, pseudo sugars, such as 5a-carba-2-D-galactopyranose, which is known to inhibit the growth of Klebsiella pneumonia (n=1), synthetic carbohydrate residues and derivatives of these (n=1) and all of the complex oligomeric permutations of these as found in nature, including high mannose oligosaccharides, the known antibiotic streptomycin (n>1).

4) A naturally occurring or synthetic organic structural motif. This term is defined as meaning an organic molecule having a specific structure that has biological activity, such as having a complementary structure to an enzyme, for instance. This term includes any of the well known base structures of pharmaceutical compounds including pharmacophores or metabolites thereof. These include beta-lactams, such as pennicillin, known to inhibit bacterial cell wall biosynthesis; dibenzazepines, known to bind to CNS receptors, used as antidepressants; polyketide macrolides, known to bind to bacterial ribosymes, etc. These structural motifs are generally known to have specific desirable binding properties to ligand acceptors.

5) A reporter element such as a natural or synthetic dye or a residue capable of photographic amplification which possesses reactive groups which may be synthetically incorporated into the oxazolone structure or reaction scheme and may be attached through the groups without adversely interfering with the reporting functionality of the group. Preferred reactive groups are amino, thio, hydroxy, carboxylic acid, carboxylic acid ester, particularly methyl ester, acid chloride, isocyanate alkyl halides, aryl halides and oxirane groups.

6) An organic moiety containing a polymerizable group such as a double bond or other functionalities capable of undergoing condensation polymerization or copolymerization. Suitable groups include vinyl groups, oxirane groups, carboxylic acids, acid chlorides, esters, amides, lactones and lactams.

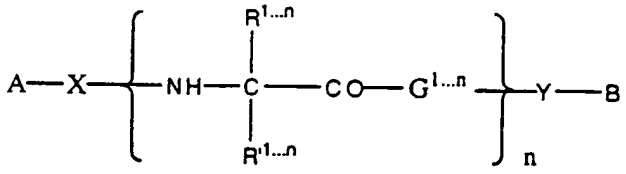
Other organic moiety such as those defined for R and R' may also be used.

7) A macromolecular component, such as a macromolecular surface or structures which may be attached to the oxazolone modules via the various reactive groups outlined above in a manner where the binding of the attached species to a ligand-receptor molecule is not adversely affected and the interactive activity of the attached functionality is determined or limited by the macromolecule. This includes porous and non-porous inorganic macromolecular components, such as, for example, silica, alumina, zirconia, titania and the like, as commonly used for various applications, such as normal and reverse phase chromatographic separations, water purification, pigments for paints, etc.; porous and non-porous organic macromolecular components, including synthetic components such as styrene-divinyl benzene beads, various methacrylate beads, PVA beads, and the like, commonly used for protein purification, water softening and a variety of other applications, natural components such as native and functionalized celluloses, such as, for example, agarose and chitin, sheet and hollow fiber membranes made from nylon, polyether sulfone or any of the materials mentioned above. The molecular weight of these macromolecules may range from about 1000 Daltons to as high as possible. They may take the form of nanoparticles (dp=100-1000Angstroms), latex particles (dp=1000-5000Angstroms), porous or non-porous beads (dp=0.5-1000 microns), membranes, gels, macroscopic surfaces or functionalized or coated versions or composites of these.

A and/or B may be a chemical bond to a suitable organic moiety, a hydrogen atom, an organic moiety which contains a suitable electrophilic group, such as an aldehyde, ester, alkyl halide, ketone, nitrile, epoxide or the like, a suitable nucleophilic group, such as a hydroxyl, amino, carboxylate, amide, carbanion, urea or the like, or one of the R groups defined below. In addition, A and B may join to form a ring or

structure which connects to the ends of the repeating unit of the compound defined by the preceding formula or may be separately connected to other moieties.

A more generalized structure of the composition of this invention is defined by the following formula:



wherein:

a. At least one of A and B are as defined above and A and B are optionally connected to each other or to other compounds;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each represents B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

As used herein, the phrase linear chain or branched chained alkyl groups means any substituted or unsubstituted acyclic carbon-containing compounds, including alkanes, alkenes and alkynes. Alkyl groups having up to 30 carbon atoms are preferred. Examples of alkyl groups include lower alkyl, for example, methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl or tert-butyl; upper alkyl, for example. cotyl, nonyl,

decyl, and the like; lower alkylene, for example, ethylene, propylene, propyldiene, butylene, butyldiene; upper alkenyl such as 1-decene, 1-nonene, 2,6-dimethyl-5-octenyl, 6-ethyl-5-octenyl or heptenyl, and the like; alkynyl such as 1-ethynyl, 2-butynyl, 1-pentynyl and the like. The ordinary skilled artisan is familiar with numerous linear and branched alkyl groups, which are within the scope of the present invention.

In addition, such alkyl group may also contain various substituents in which one or more hydrogen atoms has been replaced by a functional group. Functional groups include but are not limited to hydroxyl, amino, carboxyl, amide, ester, ether, and halogen (fluorine, chlorine, bromine and iodine), to mention but a few. Specific substituted alkyl groups can be, for example, alkoxy such as methoxy, ethoxy, butoxy, pentoxy and the like, polyhydroxy such as 1,2-dihydroxypropyl, 1,4-dihydroxy-1-butyl , and the like; methylamino, ethylamino, dimethylamino, diethylamino, triethylamino, cyclopentylamino, benzylamino, dibenzylamino, and the like; propanoic, butanoic or pentanoic acid groups, and the like; formamido, acetamido, butanamido, and the like, methoxycarbonyl, ethoxycarbonyl or the like, chloroformyl, bromoformyl, 1,1-chloroethyl, bromo ethyl ,and the like, or dimethyl or diethyl ether groups or the like.

As used herein, substituted and unsubstituted carbocyclic groups of up to about 20 carbon atoms means cyclic carbon-containing compounds, including but not limited to cyclopentyl, cyclohexyl, cycloheptyl, admantyl, and the like. such cyclic groups may also contain various substituents in which one or more hydrogen atoms has been replaced by a functional group. Such functional groups include those described above, and lower alkyl groups as described above. The cyclic groups of the invention may further comprise a heteroatom. For example, in a specific embodiment, R_2 is cycohexanol.

As used herein, substituted and unsubstituted aryl groups means a hydrocarbon ring bearing a system of

conjugated double bonds, usually comprising an even number of 6 or more (pi) electrons. Examples of aryl groups include, but are not limited to, phenyl, naphthyl, anisyl, toluyl, xylenyl and the like. According to the present invention, aryl also includes aryloxy, aralkyl, aralkyloxy and heteroaryl groups, e.g., pyrimidine, morpholine, piperazine, piperidine, benzoic acid, toluene or thiophene and the like. These aryl groups may also be substituted with any number of a variety of functional groups. In addition to the functional groups described above in connection with substituted alkyl groups and carbocylic groups, functional groups on the aryl groups can be nitro groups.

As mentioned above, R_2 can also represent any combination of alkyl, carbocyclic or aryl groups, for example, 1-cyclohexylpropyl, benzylcyclohexylmethyl, 2-cyclohexyl-propyl, 2,2-methylcyclohexylpropyl, 2,2methylphenylpropyl, 2,2-methylphenylbutyl, and the like.

d. G is a chemical bond or a connecting group and G may be different in adjacent n units; and

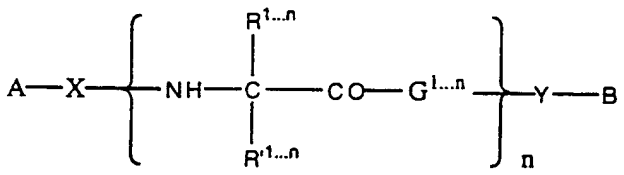
e. n is equal to or greater than 1.

Preferably, if G is a chemical bond, Y includes a terminal carbon atom for attachment to the quaternary nitrogen; and if n is 1 and X and Y are chemical bonds, R and R' are the same, A and B are different and one is other than H or R.

Under certain circumstances, A and/or B may be a chemical bond to a suitable organic moiety, a hydrogen atom, an organic moiety which contains a suitable electrophilic group, such as an aldehyde, ester, alkyl halide, ketone, nitrile, epoxide or the like, a suitable nucleophilic group, such as a hydroxyl, amino, carboxylate, aminde, carbanion, urea or the like, or one of the R groups defined below. In addition, A and B may join to form a ring or

structure which connects to the ends of the repeating unit of the compound defined by the preceding formula or may be separately connected to other moeities.

A more generalized presentation of the composition of the invention is defined by the structure



wherein:

a. At least one of A and B are as defined above and A and B are optionally connected to each other or to other compounds;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is selected from the group consisting of A, B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic, aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

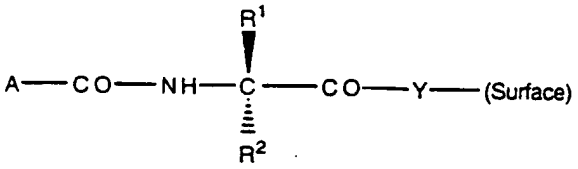
e. $n \geq 1$.

Preferably, (1) if n is 1, and X and Y are chemical bonds, A and B are different and one is other than a chemical bond, H or R; (2) if n is 1 and Y is a chemical

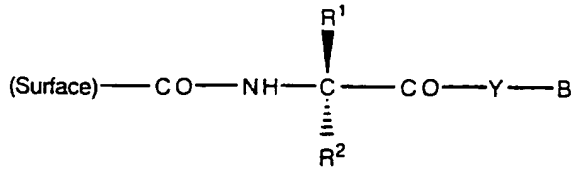
bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group and G-B is other than an amino acid residue or a peptide; (3) if n is 1 and X, Y, and G each is a chemical bond, A and B each is other than a chemical bond, an amino acid residue or a peptide; and (4) if n is 1, either X or A has to include a CO group for direct. connection to the NH group.

These compositions may be used to mimic various compounds such as peptides, nucleotides, carbohydrates, pharmaceutical compounds, reporter compounds, polymerizable compounds or substrates.

In one embodiment of the invention, at least one of A and B represents an organic or inorganic macromolecular surface functionalized with hydroxyl, sulfhydryl or amine groups. Examples of preferred macromolecular surfaces include ceramics such as silica and alumina, porous or nonporous beads, polymers such as a latex in the form of beads, membranes, gels, macroscopic surfaces, or coated versions or composites or hybrids thereof. A general structure of a chiral form of these materials is shown below:

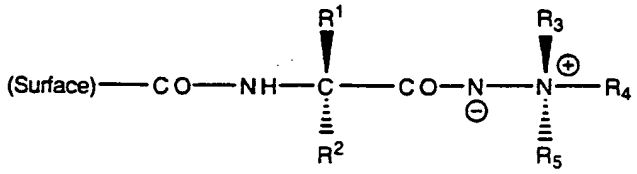


In another embodiment of the invention, the roles of A and B in the structure above are reversed, so that B is a substituent selected from the list given above and A represents a functionalized surface as shown for one of the enantiomeric forms:

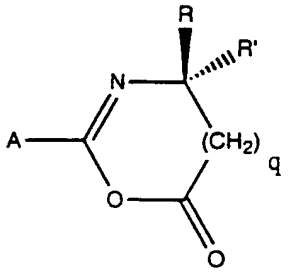


In the description that follows, R^n where n = an integer will be used to designate a group from the definition of R and R^1.

In a preferred embodiment, group A or B in the above structure is an aminimide moiety. This moiety may be introduced, for example by reacting the oxazolone with an asymmetrically substituted hydrazine and alkylating the resulting hydrazide, (e.g., by reaction with an alkyl halide, or epoxide). An example of such a surface is shown below.

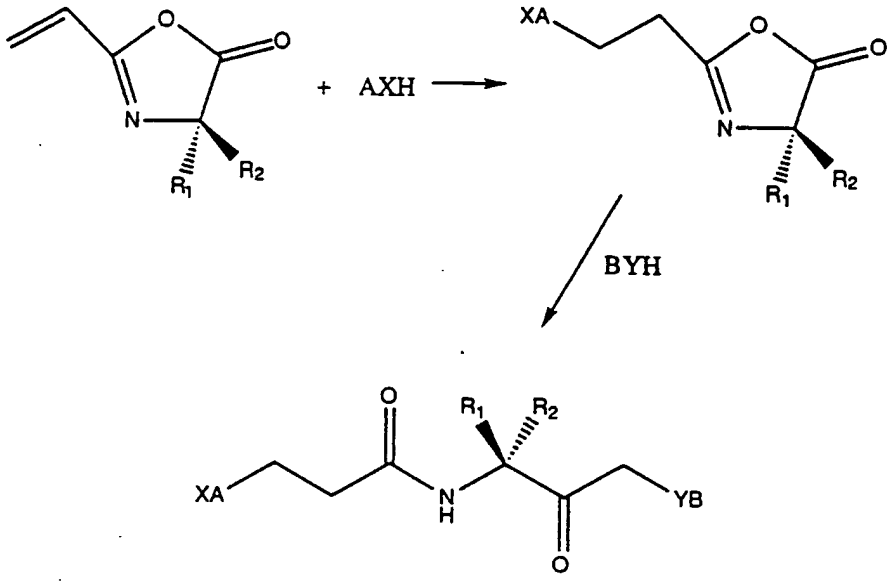


Another embodiment of the invention relates to an oxazolone ring having the structure



where A, R and R' are as described above and q is zero or 1. Preferably, Y is a chemical bond This ring is useful for preparing the desired oxazolone derivatives.

A further embodiment of the invention exploits the capability of oxazolones with suitable substituents at the 2-position to act as reactive agents. Appropriate substituents include vinyl groups, which make the oxazolone a Michael acceptor, haloalkyl and alkyl sulfonate ester and epoxide groups. For example, Michael addition to the double bond of a chiral 2-vinyloxazolone followed by a ring opening reaction results in a chiral conjugate structure. This general reaction scheme, illustrated for the case of a 2-vinyl azlactone derivative, is as follows:



wherein X can represent a sulfur, oxygen or nitrogen atom; Y can represent a sulfur, oxygen, or nitrogen atom; and substituents A and B, as described above, may adopt a variety of structures, differing markedly in their physical or functional properties or being the same, may be chiral or achiral, and may be preferably selected from amino acids, oligopeptides, polypeptides and proteins, nucleotides, oligonucleotides, ligand mimetics, carbohydrates, aminimides, structures found in therapeutic agents, metabolites, dyes, photographically active chemicals, or organic molecules having desired steric, charge, hydrogen-bonding or hydrophobicity characteristics, or containing polymerizable vinyl groups.

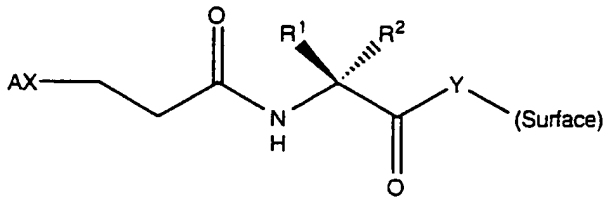
The Michael reaction described above is usually carried out using stoichiometric amounts of the nucleophile, AXH, and the oxazolone in a suitable solvent, such as toluene, ethyl acetate, dimethyl formamide, an alcohol, or the like. The product of the Michael addition is preferably isolated by evaporating the reaction solvent *in vacuo* and purifying the material isolated using a technique such as recrystallization or chromatography. Gravity- or pressure-chromatography, on one of a variety of supports, e.g., silica, alumina, under normal- or reversed-phase conditions, in the presence of a suitable solvent system, may be used for purification. The selectivities of the Michael and oxazolone ring-opening processes impose certain limitations on the choice of AXH and BYH nucleophiles shown above. Specifically, nucleophiles of the form ROH tend to add primarily via the ring-opening reaction, and usually require. acidic catalysts (e.g., BF3); thus, X should not normally be oxygen.

Likewise, primary amines tend to add via ring-opening, and X should therefore not be NH. Secondary amines readily add to the double bond under appropriate reaction conditions, but many can also cause

ring-opening; accordingly, X or Y can be N, provided A or B are not hydrogen. Nucleophiles of the form RSH will exclusively add via ring-opening if the sulfhydryl group is ionized, *i.e.*, in the presence of a (non-oxazolone-reactive) base strong enough to remove the SH proton; on the other hand, such sulfur containing nucleophiles will exclusively add via Michael reaction under non-ionizing, i..e., neutral or mildly acidic conditions. During the Michael addition, it is important to limit the presence of hydroxylic species in the reaction mixture (e.g., moisture) to avoid ring-opening side-reactions.

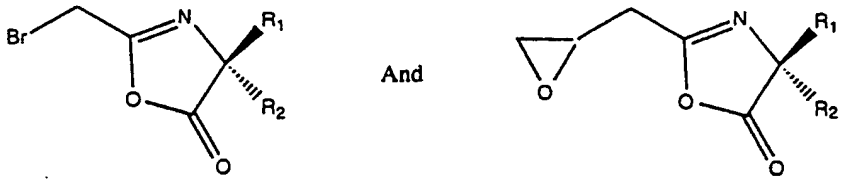
Summarizing, AXH can be a secondary amine or a thiol, and BYH can be a primary or secondary amine, a thiol, or an alcohol.

In one variant of the Michael-ring-opening sequence given above, A is a substituent selected from the foregoing list and BXH comprises an organic or inorganic macromolecular surface, e.g., a ceramic, a porous or nonporous bead, a polymer such as a latex in the form of a bead, a membrane, a gel or a composite, or hybrid of these; the macromolecular surface is functionalized with hydroxyl, sulfhydryl or amine groups which serve as the nucleophiles in the ring-opening reaction. The reaction sequence is carried out under conditions similar to those given for the nonpolymeric cases; purification of the final product involves techniques used in the art to purify supports and other surfaces after derivatization, such as washing, dialysis, etc. The result of this reaction sequence is a structure such as the one shown below:



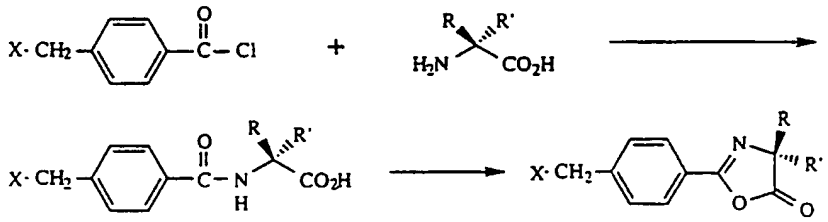
In another variant, the roles of AXH and BYH are reversed, so that BYH is the substituent selected from the list above and AXH represents a functionalized surface.

Other important bifunctionally reactive oxazolone derivatives include:



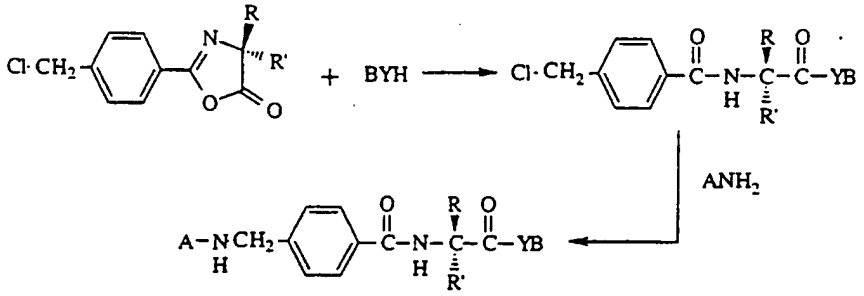
These are produced by acylation of an alpha,alpha-disubstituted amino acid residue with the apropriate functionalized acid chloride, followed by cyclization to the oxazolone.

Alternatively, oxazolones posessing reactive groups at the 2-position may be produced*via* suitable acylation reactions, as shown for the specific example of a benzoyl chloride oxazolone derivative containing a reactive p-benzyl group:

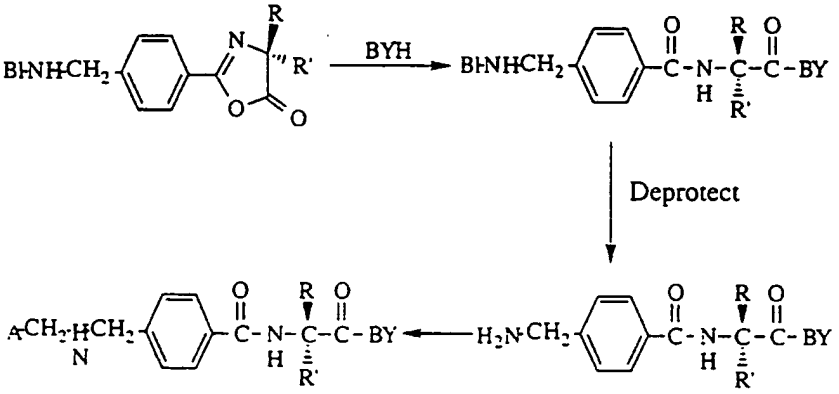


In the case where X is part of a group whose reactivity is orthogonal to that of the oxazolone ring, such as in the case of a benzyl chloride group, ring-opening addition with BYH may be carried out and followed by reaction

with an appropriate AXH group, e.g. a primary amine, to give the product shown:



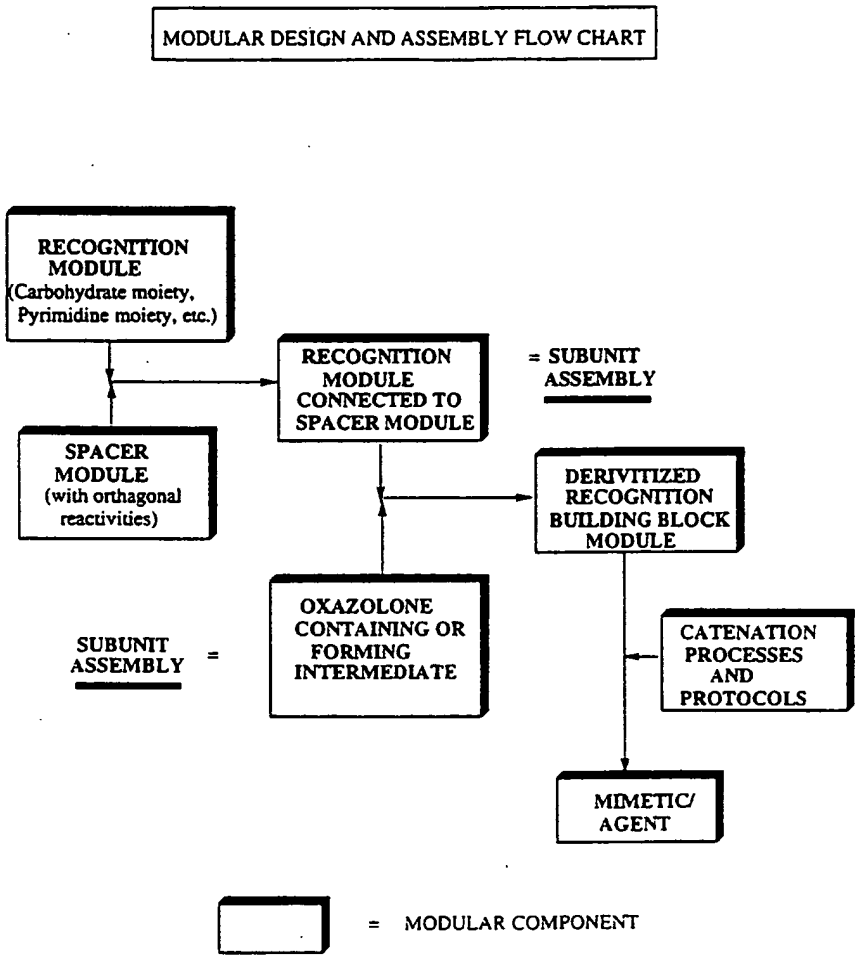
If in the above sequences the benzylic electrophile competes with the oxazolone ring for the nucleophile BYH, a suitable protecting group, shown as Bl below, may be used to block the benzylic electrophile. Subsequent to the ring-opening addition of BYH, the protected group is removed using standard techniques (e.g., if the protecting group is Boc, it is removed by using dilute TFA in CH2Cl2), and the resulting product is then reacted with an appropriate electrophile, e.g., A-CH_2-Br, thus introducing substituent A into the molecule.



4.3.2 Catenation of Chiral Oxazolones Producing Chiral Oligomers and Polymers

By choosing oxazolone-derived building blocks possessing functional groups capable of establishing predictable binding interactions with target molecules, and using synthetic techniques such as those broadly described above to effect catenation (linking) of the building blocks, it is possible to construct sequences of oxazolone-derived subunits mimicking selected native oligomers or polymers, e.g. peptides and polypeptides, oligonucleotides, carbohydrates as well as any other biologically active species whose three dimensional binding geometry can be mimicked by various combinations of oxazolone derivitive containing scaffolds and side chains. This may be accomplished using a wide variety of side chain recognition group substituents, including, but not limited to the substituents found in the side chains of naturally occuring amino acids; purine and pyrimidine groups as well as derivatives and variants of these; natural and synthetic carbohydrate recognition groups, such as sialic acids; groups containing organic structures with known pharmacological activities, such as beta lactam antibiotic moities, which are known to be efficient inhibitors of bacterial cell wall biosynthesis, to produce structures which have highly specific activities. These moities may be attached, arranged and spaced in a position-specific manner along a scaffold whose basic geometry, spacing, rigidity and other properties can be designed and locally tuned to functionally mimic the natural scaffolds found in peptides, proteins, oligonucleotides or carbohydrates; or which can simply serve to array sequences or combinations of these side chain recognition groups in appropriate structural relationships to the scaffold and to each other to produce species with highly specific and selective activity. In addition. because of the improved hydrolytic and enzymatic stability properties of the oxazolone-derived

linkages, these designed functional molecules will have better stability and pharmacokinetic properties than those of the native species. The integrated modularity of the chemistries allows the construction of this wide variety of molecules to be carried out in a manner analogous to the design of an electronic device by combining component subsystems using a relatively small number of interchangable reactive modules and protocols. This is figuratively outlined below:

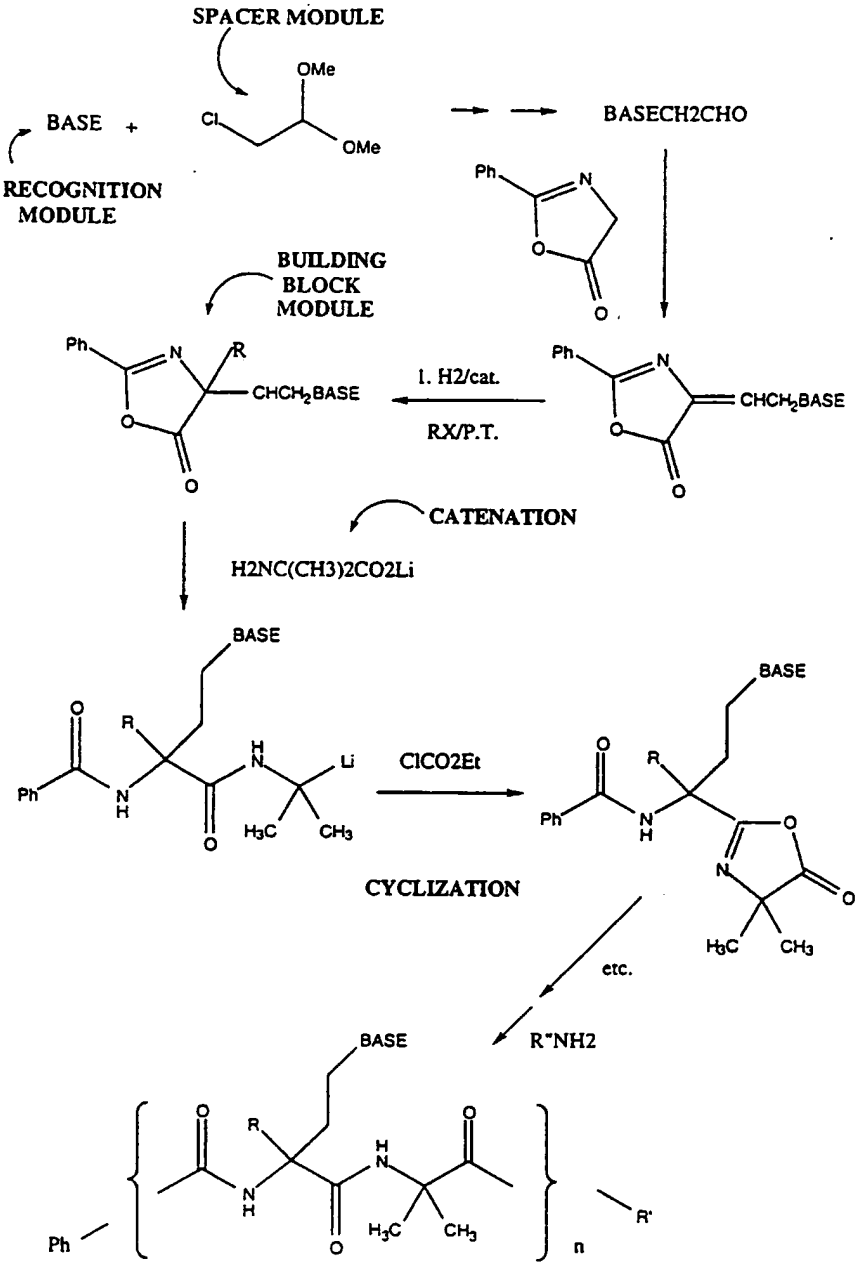


This approach is illustrated below for the introduction of a generic "base" (purine or pyrimidine) group into an oxazolone-derived scaffold connected via a carbonyl-

terminal spacer. While the example uses a base as the recognition group, it should be kept in mind that this group can be any group which will provide the desired end product, such as, for example, a carbohydrate, a pharmacophore moiety or a designed synthetic recognition element.

The following specific sequence illustrates the construction of a ligand having bases attached to every other oxazolone-derived module. Alternatively, species may be constructed with bases attached to each sequential oxazolone module. The substituents on the recognition group-bearing modules may all posess the same chirality, may have regularly alternating chirality or may be racemic, depending on the desired structural relationships between the individual recognition groups and between each recognition group and the backbone scaffold.

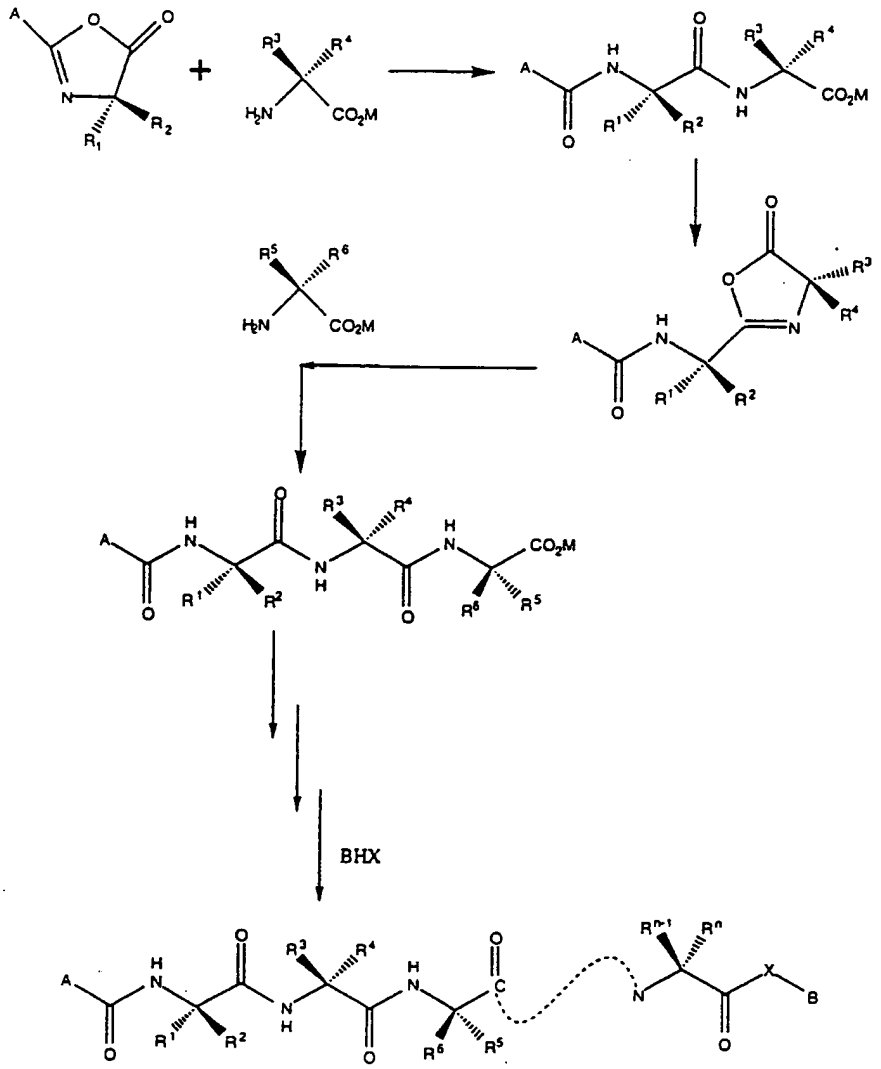
Alternatively, Other variations may be constructed, including those employing non-hydrogenated modules, which produces derivatives with the bases attached to the scaffold via double bonds. In these structures, the assembled ligand may be subjected to multiple simultaneous stereospecific hydrogenation of this unsaturated linkage producing derivatives with alpha-hydrogen substituents in a stereocontrolled manner, and avoiding the racemization problems involved in constructing these ligands via alpha- hydrogen containing oxazolones. as outlined above.


SPACER MODULE
OMe
Cl
OMe
BASE +
RECOGNITION MODULE
BASECH2CHO
Ph
N
O
O
BUILDING BLOCK MODULE
Ph
N
R
CHCH2BASE
O
O
1. H2/cat.
RX/P.T.
Ph
N
CHCH2BASE
O
O
CATENATION
H2NC(CH3)2CO2Li
BASE
O
R
Ph
N
H
H
N
Li
O
H3C
CH3
ClCO2Et
BASE
O
R
Ph
N
H
O
N
O
H3C
CH3
CYCLIZATION
etc.
R"NH2
BASE
O
R
N
H
H
N
O
H3C
CH3
O
Ph
n
R'

4.3.2.1 Catenation Via Alternating Sequences of Nucleophilic Oxazolone-Ring-Opening Addition Reactions Followed by Oxazolone-Forming Cyclization Reactions

a. Alpha,Alpha-Disubstituted Sequences

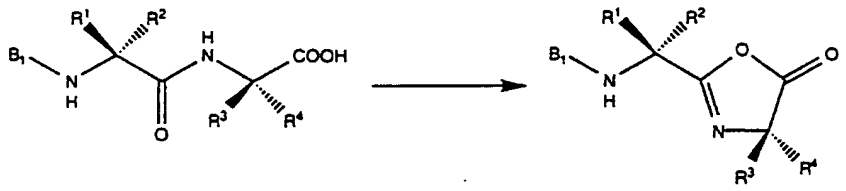
According to this approach, oxazolone modules are catenated via ring-opening nucleophilic attack by the amino group of a (chiral) alpha,alpha-disubstituted amino acid derivative, usually a lithium salt; the resulting adduct is subsequently recyclized to form a terminal oxazolone (with retention of chirality). This oxazolone is then subjected to another nucleophilic ring-opening catenation reaction sequence, producing a growing chiral chain, as shown below. This procedure is repeated until the desired polymer is obtained.



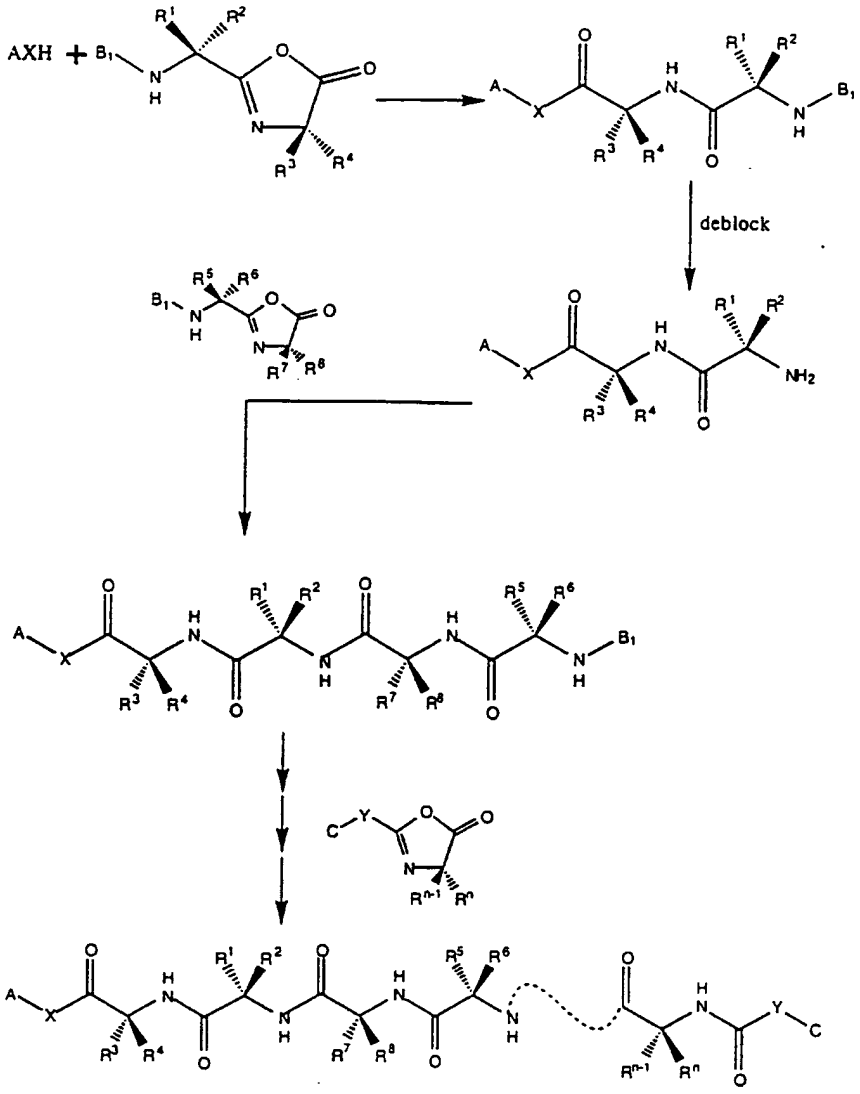
Wherein M is an alkali metal; each member of the substituent pairs R^1 and R^2, R^3 and R^4, R^5 and R^6 and R^n and R^{n-1} differs from the other and, taken alone, each signifies alkyl, cycloalkyl, or substituted versions thereof, aryl, aralkyl or alkaryl, or substituted and heterocyclic versions thereof; these substituent pairs can also be joined into a carbocyclic or heterocyclic ring; preferred forms of R1 and R2 are the side chain substituents occuring in native polypeptides, oligonucleotides, carbohydrates,

pharmacophores, variants or mimetics of these, or any other side chain substituent which can be attached to a scaffold or backbone to produce a desired interaction with a target system.; X represents an oxygen, sulfur, or nitrogen atom; and A and B are the substituents described above.

A chiral oxazolone derivative containing a blocked terminal amino group may be prepared from a blocked, disubstituted dipeptide, that was prepared by standard techniques known to those skilled in the art, as shown:



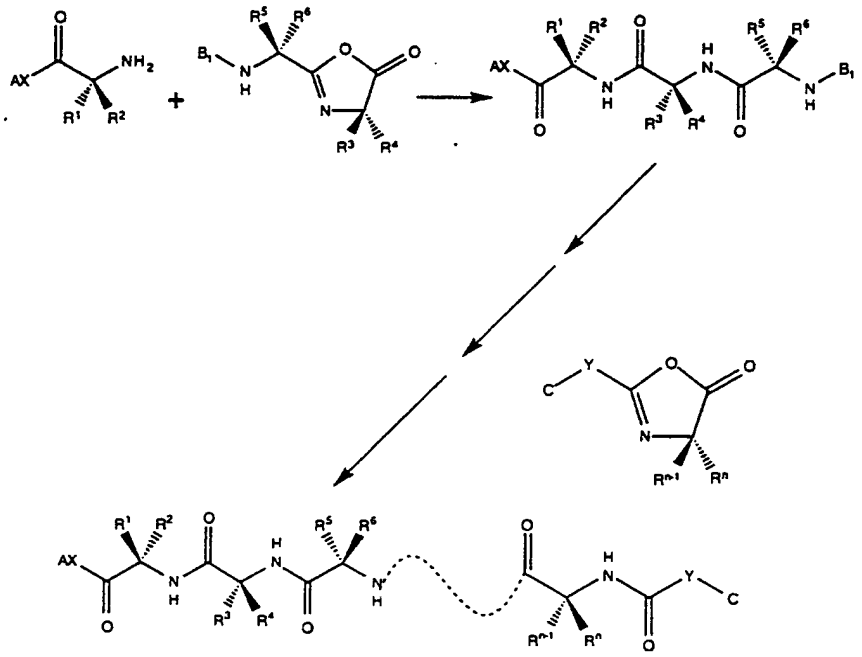
wherein B_1 is an appropriate protecting group, such as Boc (t-butoxycarbonyl) or Fmoc (fluorenyl- methoxy carbonyl). One may then use this oxazolone to acylate an amine, hydroxyl, or sulfhydryl-group in a linker structure or on a functionalized solid support, represented generically by AXH, using the reaction conditions described above. This acylation is followed by deblocking, using standard amine deprotection techniques compatible with the overall structure of the amide (i.e., the amine protecting group is reaactively orthogonal with respect to any other protecting or functional groups that may be present in the molecule), and the resulting amino group is used for reaction with a new bifunctional oxazolone, generating a growing chiral polymeric structure, as shown below:



In the reaction shown, Y is a linker, such as, for example, a functionalized aryl group; X is a nitrogen of suitable structure, an oxygen or a sulfur atom; each member of the substituent pairs R^1 and R^2, R^3 and R^4, R^{n-1} and R^n differs from the other and, taken alone, each signifies alkyl, carbocyclic, or functionalized versions thereof, aryl, aralkyl or alkaryl or functionalities, including heterocyclic versions thereof, preferred forms of R1 and R2 are the side-chain substituents occuring in native polypeptides,

oligonucleotides, carbohydrates, pharmacophores, variants or mimetics of these, or any other side-chain substituent which can be attached to a scaffold or a backbone to produce a desired interaction with a target system; substituent R can also be part of a carbocyclic or heterocyclic ring; A is a substituent as described above; C. is a substituent selected from the set of structures for A; and B_1 is a blocking or protecting group.

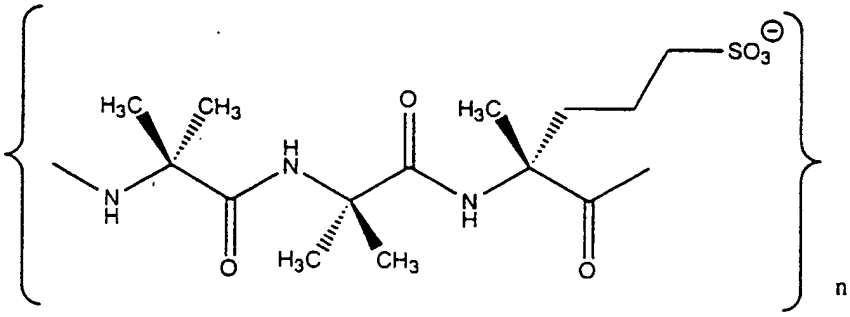
It can be seen that the above catenation involves introduction of two amino acid residues per polymer-elongation cycle and therefore produces ligands with an even number of residues. To obtain ligands containing an odd number of residues, a preliminary step may be carried out with a suitable amino acid derivative as shown below, prepared via standard synthesis.



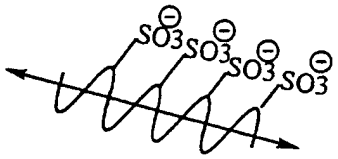
In the polymers described above each individual module may carry a recognition group substituent, as is the case for ligands designed to mimic peptides. Alternatively, this sequence of reactions can be

used to construct ligands with continuously variable stepwise control of the periodicity and the stereochemistry of each of the attached substituent groups and, consequently, of the resulting structural and functional properties of the ligand. This can be done by separating the recognition group-bearing modules from each other by one or more modules which do not carry recognition groups. These intervening modules may be achiral, alpha, alpha-disubstituted or, in cases where chirality is not important, they may be standard hydrogen-bearing alpha amino acid modules. These may serve as spacers, to regulate the periodicity of substitution or may serve various other co-functions, such as limiting the flexibility of the ligand. The substituents on the recognition group-bearing modules may be constructed to all posess the same chirality, may have regularly alternating chirality or may be racemic, depending on the desired structural relationship between the individual recognition groups and between each recognition group and the backbone scaffold.

Alternatively, modular "sub assemblies" capable of conferring higher order structural properties may be pre- constructed and assembled together using these same reaction sequences in a manner which allows control of the higher order structure. This is illustrated for the case of a polymer formed witha repeating pattern of alternating modules of the type:



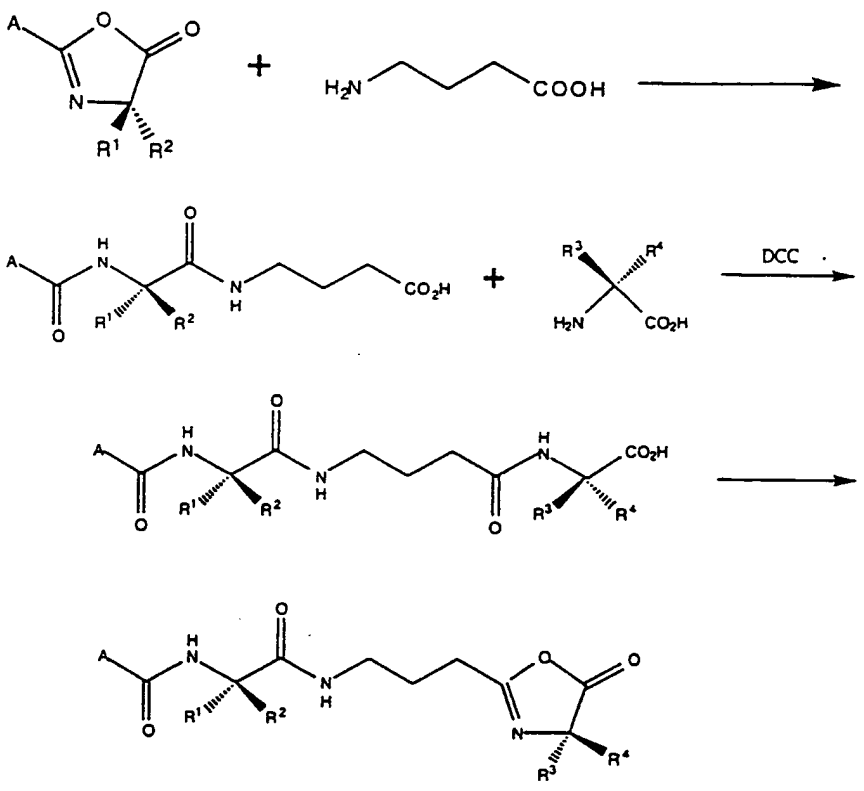
This polymer will form 3-10 helices, driven by the conformational restrictions imposed by the repetitive viscinal disubstitution. This triadic periodicity results in the formation of a helical superstructure which has charged sulfonate groups lined up regularly along one side of the helix:



This helix-forming phenomenon has been observed with naturally occuring peptides which contain sequences of adjacent aminoisobutyric acid (aib) residues, a naturally occuring achiral alpha, alpha-disubstituted amino acid. Examples of this include certain naturally occuring peptide antibiotics, such as alemethicin and suzukicillin.whose aib-derived helical structures have been postulated to be an important constituent in the cell wall-disrupting mode of action of these antibiotics.

b. Other Bifunctionally Reactive Elements

At any point in the polymer syntheses shown above, a structural species, possessing (1) a terminal OH, -SH or $-NH_2$ group capable of ring-opening addition to the oxazolone and (2) another terminal group capable of reacting with the amino group of a chiral alpha, alpha'disubstituted amino acid, may be inserted in the polymer backbone as shown below;

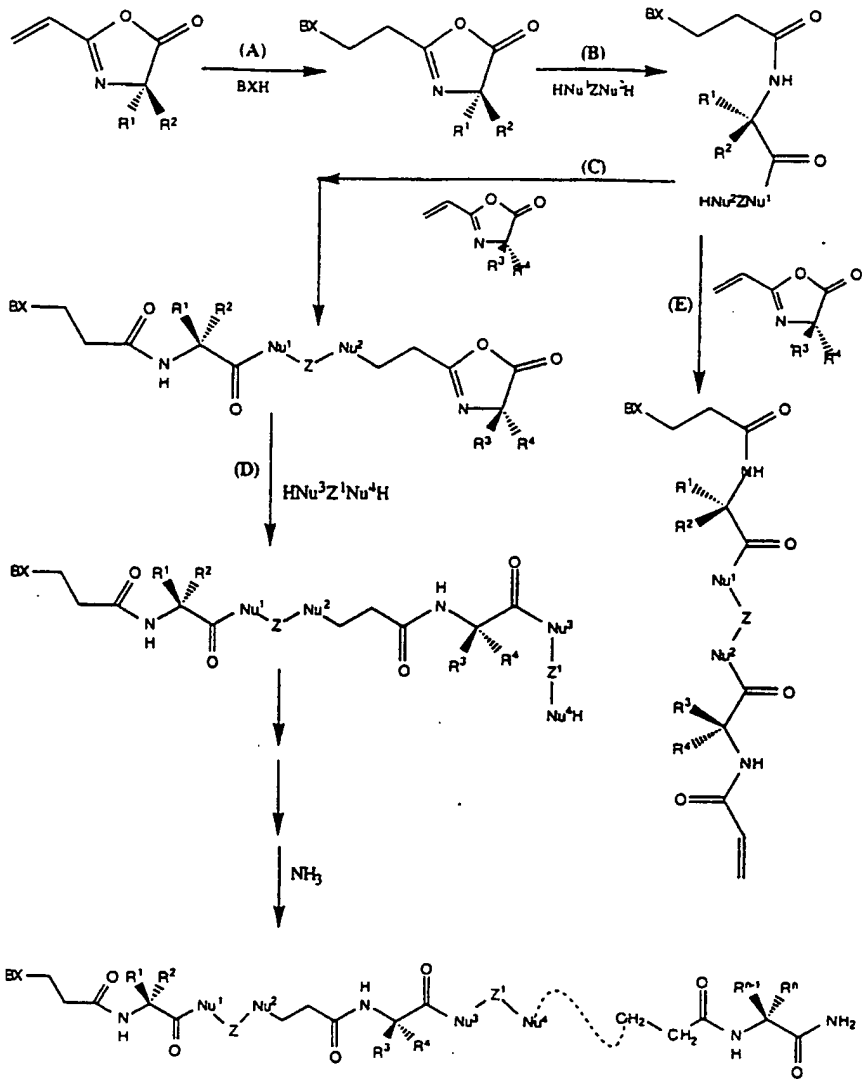


This process may be repeated, if desired, at each step in the synthesis where an oxazolone ring is produced. The bifunctional species used may be the same or different in each individual step of the synthesis.

The experimental procedures described above for oxazolone formation and for the use of oxazolones as acylating agents are expected to be useful in these oxazolone-directed catenations. Solubility and coupling problems that may arise in specific cases can be dealt with effectively by one with ordinary skill in the art of polypeptide and peptide mimetic synthesis. For example. special solvents such as dipolar aprotic solvents (e.g., dimethyl formamide, DMF, dimethyl sulfoxide, DMSO, N-methyl pyrolidone, etc.) and chaotropic (molecular aggregatebreaking) agents (e.g., urea) will be very useful as catenations produce progressively larger molecules.

4.3.2.2 Catenations Using Bifunctionally Reactive Oxazolones

When the substituent at the 2-position of the oxazolone (azlactone) ring is capable of undergoing an addition reaction that proceeds with retention of the chirality at the 4-position, this addition reaction may be combined with a ring-opening acylation to produce chiral polymeric sequences. This is shown for the case of alkenyl azlactones
below.



In the above sequence of reactions, A denotes a structure of the form described above and HNu^1-Z-Nu^2H represents a structure containing two differentially reactive nucleophilic groups, such as methylamino-ethylamine, 1-amino propane-3-thiol, and so on; groups Nu^1, Nu^2, Nu^3 and Nu^4 need not be identical and Z is a linker structure as described above.

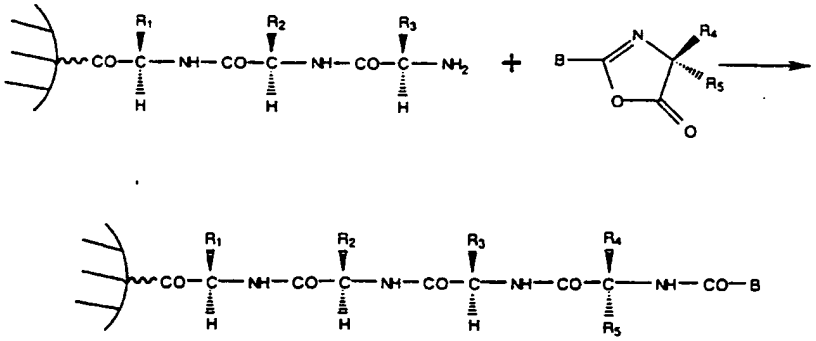
Structure HNu^1-Z-Nu^2H may contain two nucleophilic groups of differential reactivity, as stated above, or if Nu^1 and Nu^2 are of comparable reactivity one of the nucleophilic groups is protected to prevent it from competing with the other and is deprotected selectively following acylation; protecting groups commonly used in the art of peptide synthesis (e.g., for the nucleophilic groups such as amino, hydroxyl, thio, etc.) are useful in the protection of one of the Nu substituents of the structure HNu^1-Z-Nu^2H. The product of the acylation reaction with HNu^1-Z-Nu^2H (after Nu-deprotection, if necessary) is further reacted with a new oxazolone unit in a Michael fashion, and this addition is followed by ring-opening acylation with an additional dinucleophile; repetition of this sequence of synthetic steps produces a growing polymeric molecule. Reaction conditions for carrying out these processes are similar to those described above for related polymers.

The above types of oligomers are highly useful biochemically because of their structural similarity to biological scaffolds, particularly polypeptide scaffolds. The substituents R can be chosen to tailor the steric, charge or hydrophobicity characteristics of the oligomer such that a versatile mimetic results.

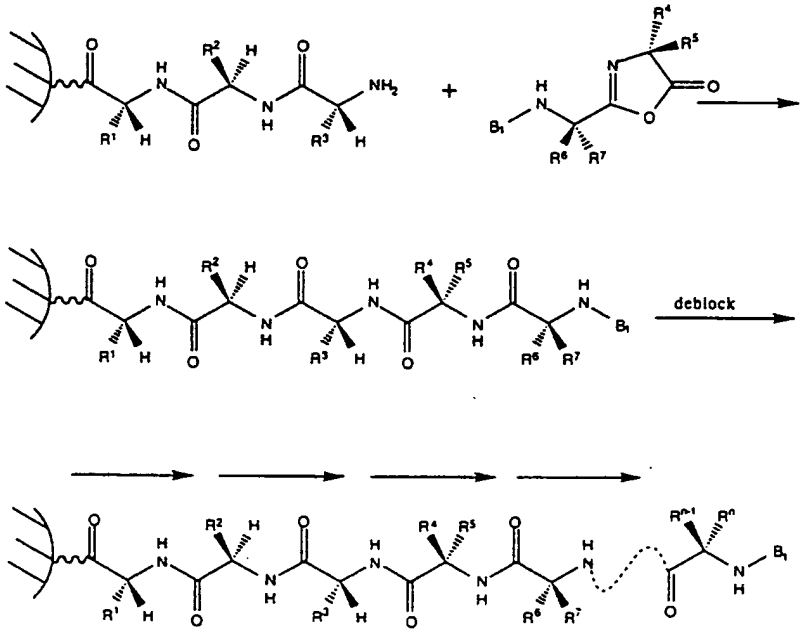
4.3.3 Functionalization of Peptides and Proteins Using Oxazolones

In a further embodiment of the invention, the nucleophilic ring-opening of asymmetrically disubstituted oxazolones may be utilized to introduce a chiral residue or sequence in selected positions in peptides or proteins to produce hybrid molecules with improved hydrolytic and enzymatic stability properties.

The reaction of a chiral azlactone with the amino terminus of a synthetic tripeptide attached to a Merrifield support is shown below.

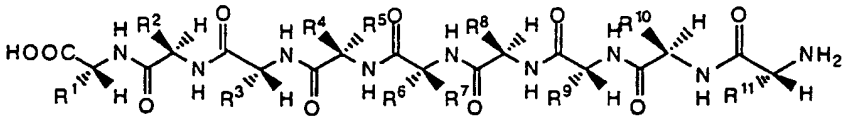


The oxazolone used in the above aminolysis may contain a blocked amino terminus which, after the aminolysis, is deblocked and used for further elongation via acylation. This synthetic variation is shown below (B_1 stands for a suitable blocking group as described above).



After the desired oxazolone units have been used to elongate a given polypeptide, the polypeptide synthesis may be continued, if desired, using standard peptidesynthesis techniques.

The structure below illustrates a short polymer containing nine subunits prepared as above and detached from the solid phase synthesis support.

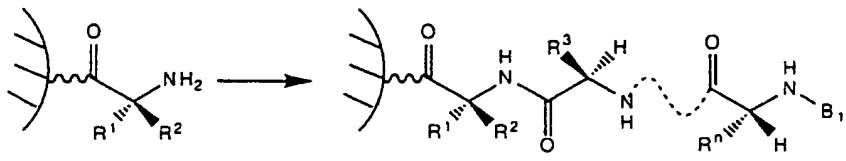


In the polyamide structure shown above, each of the R groups signifies alkyl, carbocyclic, or substituted versions thereof; aryl, aralkyl, alkaryl, or substituted versions thereof, including heterocyclic versions; the R groups can also define a carbocyclic or heterocyclic ring; preferred structures for the R groups in this application are those mimicking the structures of the side-chains of naturally-occurring amino acids.

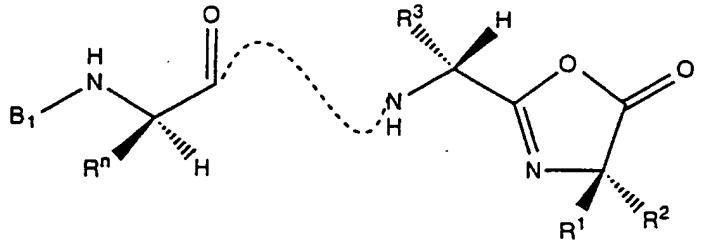
The syntheses outlined above may be carried out using procedures similar to those described previously for related molecules and macromolecules.

Alternatively, disubstituted chiral azlactones may be utilized to introduce a variety of novel, unnatural residues into peptides or proteins using the following multistep procedure:

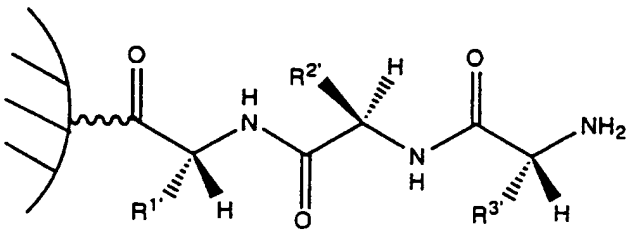
a. Synthesis of a peptide whose carboxyl terminal residue is chiral and disubstituted, preferably via solid phase synthesis:



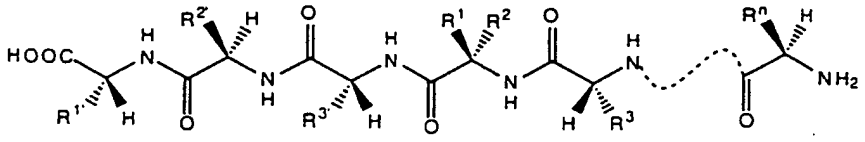
b. Detachment of the peptide prepared by solid phase synthesis from the support, with reblocking of the N-terminus if necessary, followed by cyclization producing the oxazolone as shown below:



c. Synthesis of a second desired peptide sequence on a solid support:



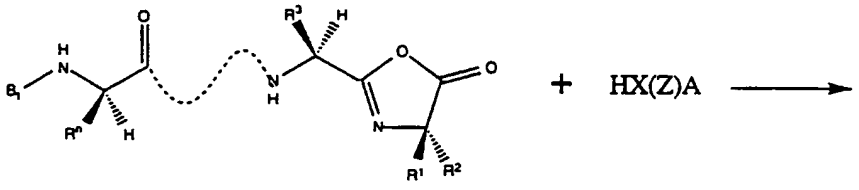
d. Coupling of the peptides produced in steps (b) and (c) above, under suitable reaction conditions, producing a novel peptide containing unnatural residues, shown below after detachment of the peptide from the support and removal of all protecting groups used during its synthesis.

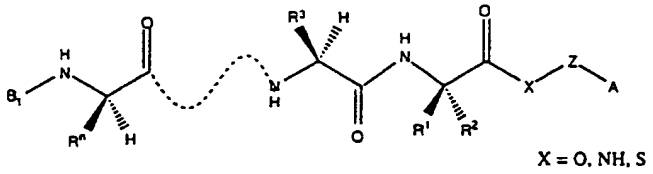


In the structure above, each of the R groups signifies alkyl, cycloalkyl, aryl, aralkyl or alkaryl, or substituted or suitably heterocyclic versions thereof; the R groups may also define a carbocyclic or heterocyclic ring; preferably the R groups are structural mimetics of the side-chains of naturally-occurring amino acids.

\ Again, the reactions shown in steps a-d above are carried out using the conditions described above for related cases. Couplings of peptide segments on a support or in solution are carried out using the traditional techniques from the field of peptide synthesis.

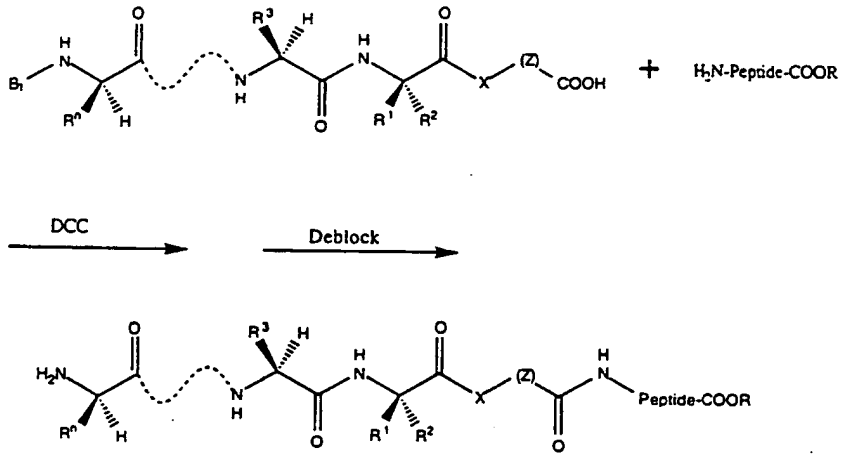
In a variation of the above synthesis, the oxazolone peptide produced in step (b) above may be reacted with a variety of bifunctional nucleophilic molecules to give acylation products as shown below:



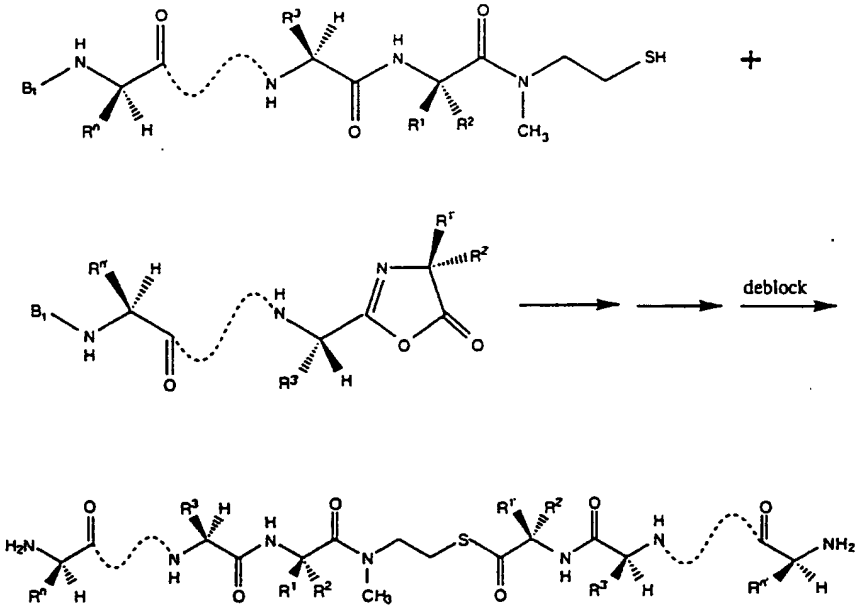


The above acylation product may be coupled with a peptide to produce novel chiral hybrids; two coupling routes may be used.

(1) If A is a group which can be condensed with an amino group, the condensation reaction is used for coupling. For example, if A is a carboxyl group, condensation with a peptide amine using DCC or a similar reagent produces the desired product. Reaction conditions and suitable (orthogonal) protecting groups well-known in the art, such as those described above, are expected to be useful.



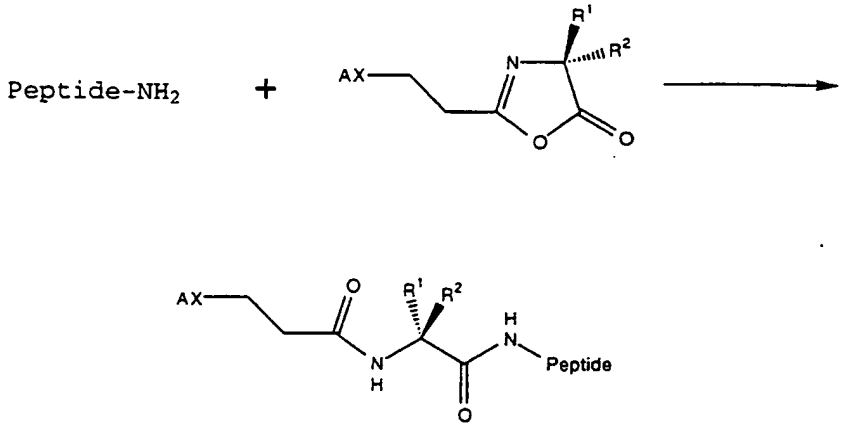
(2) If A is a suitable nucleophilic group (e.g., hydroxyl, amino, thio, etc.) it may be used to open a peptide oxazolone containing a protected amino terminus. In the case shown below, groups Y, A and Z of the general structure shown above have been defined as follows: Y = NCH_3, A = SH and Z = CH_2CH_2:



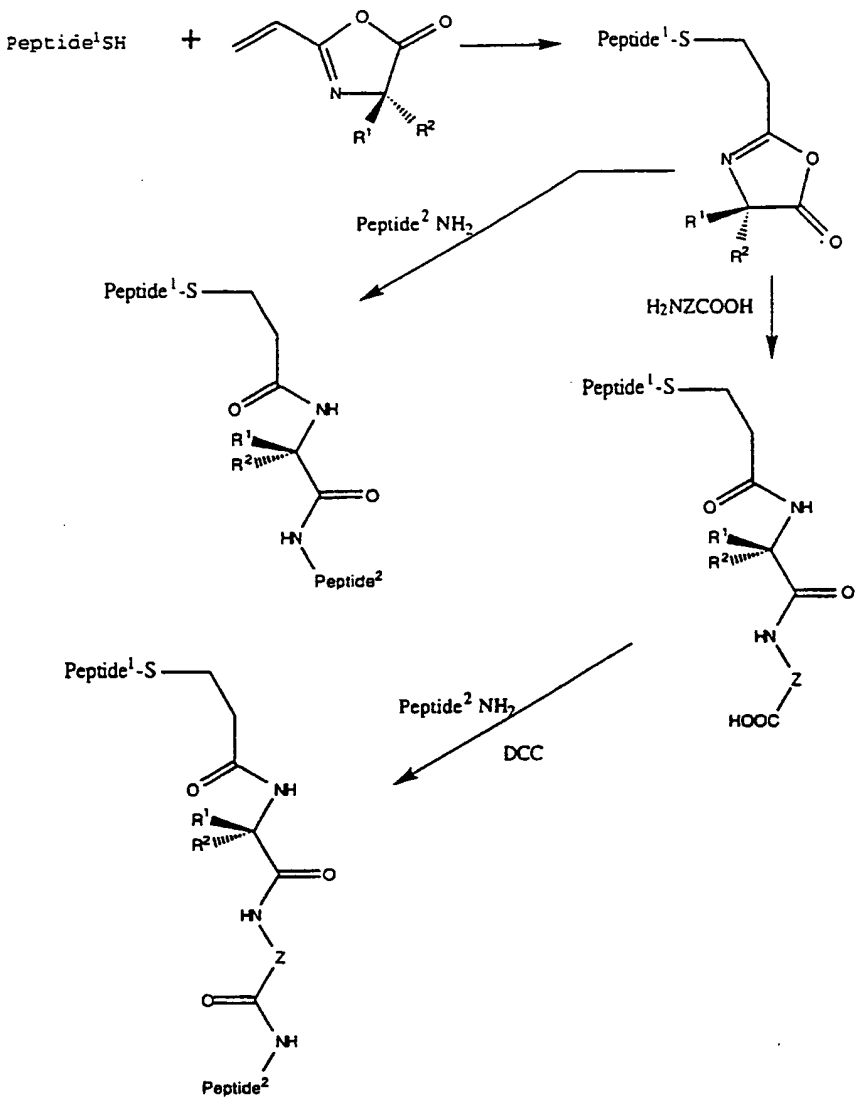
The above reactions are run under conditions, similar to those described above for related peptide syntheses. A great variety of molecules possessing nucleophilic hydroxyl, thio, amino and other groups, e.g., carbohydrates, may be conjugated with peptidic and related frameworks using reactions with suitable oxazolones as outlined above.

Alternatively, residues may be attached to or inserted into peptide chains using oxazolones with reactive groups attached at the 2-position of the ring. This may be accomplished in either of two ways, as illustrated below for the case of 2-alkenyl azlactones.

(1) Nucleophilic attack on an azlactone, that was previously derivatized via a Michael addition using a nucleophile of general structure AXH, with a peptide amine:



(2) Michael addition of a peptide nucleophile, e.g., a sulfhydryl group, to the double bond of a 2-vinyl oxazolone, followed by nucleophilic attack on the oxazolone ring by another peptide nucleophile, e.g., an amine followed by further modifications; this sequence produces polymeric molecules of a variety of structures as shown below:

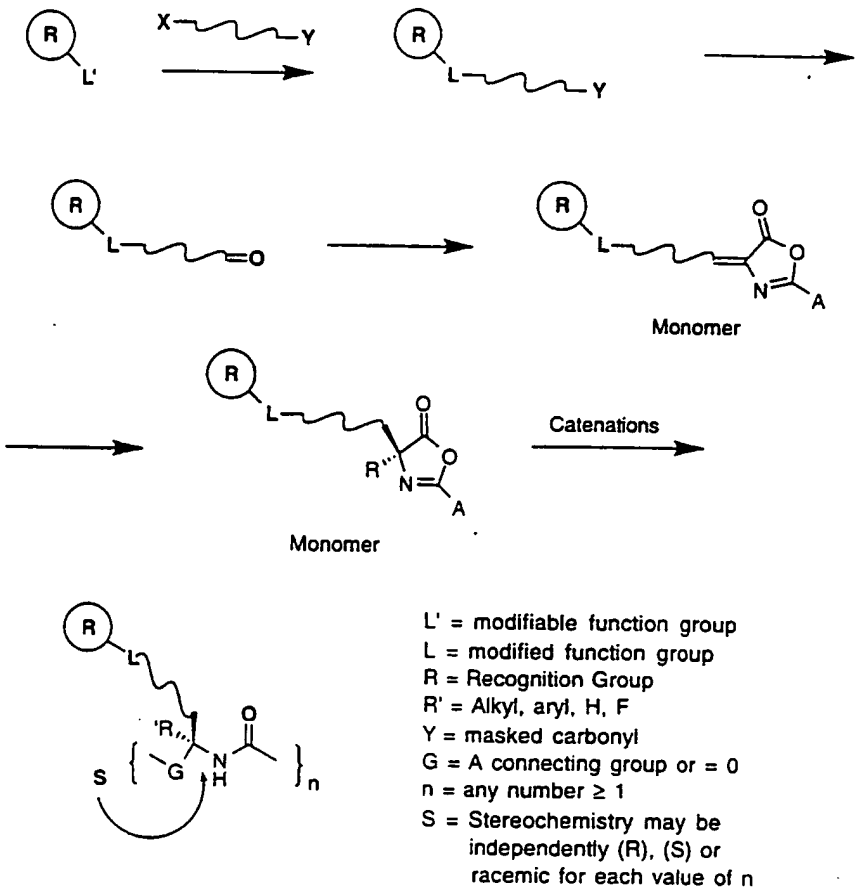


4.3.4. Other Mimetics

Oxazolone-derived mimetics can be produced, using the oxazolone-forming and catenation chemistries outlined above, so as to produce backbones having natural or synthetic recognition groups, such as purine or pyrimidine bases, carbohydrates, pharmacophores, etc., attached as side chain

substituents via appropriate spacers, i.e. R or R' in the general structural formulas described above represents a recognition group-spacer sub assembly.

This may be accomplished, for example, via the following general synthesis scheme:



4.3.4.1. Synthesis of Oligonucleotide Mimetics

As discussed previously, much attention has been focused on the construction and application of molecules which possess the property of binding to nucleic acids. In the course

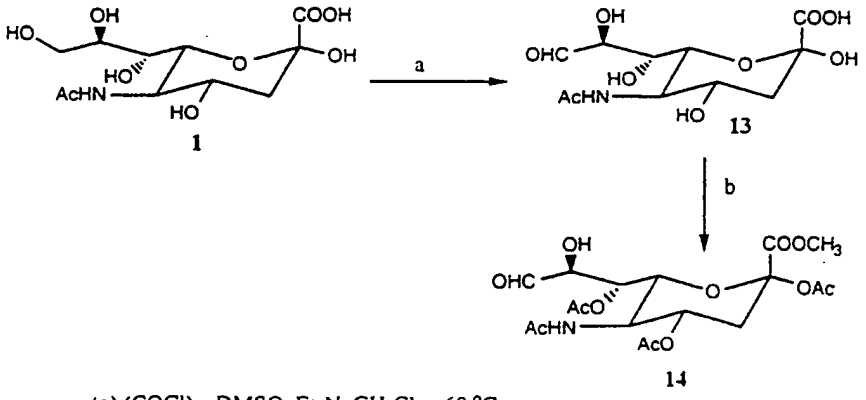
of work in this area, a great amount of knowledge has been amassed vis-a-vis 1.) the ability of a synthetic scaffolding to support a series of natural or designed bases in such a manner that tight binding to natural nucleic acids is observed; 2.) the requirements for designed or naturally occuring bases other than guanocine, cytosine, thymidine, adenosine or uridine to efficiently bind (hybridize) to another natural base or nucleotide. It has been demonstrated that even unnatural or modified bases can show efficient hybridization if projected from an effective scaffold. Our strategy, disclosed herein, is to append natural and/or unnatural bases (e.g. thymine, guanidine, 5-fluorouricil(5FU)) onto oxazolone backbones to form an antisense strand, or nucleotide mimetic. The resulting linkages and backbones are superior in their resistance to base, acid and proteolytic/phospholytic activity. The bases can be attached using appropriate spacers and the stereochemistry and periodiocity of substitution geometry and rigidity of the backbone scaffold can be designed such that the bases are arrayed and projected in space to provide the optimum arrangement and orientation of the bases to hybridize with their targeted counterparts. Specific examples of the synthesis of oxazolone-derived oligonucleotide mimetics are given below.

4.3.4.2. Synthesis of Carbohydrate Mimetics

As mentioned previously, carbohydrates increasingly are being viewed as the components of living systems with the enormously complex structures required for the encoding of the massive amounts of information needed to orchestrate the processes of life, e.g., cellular recognition, immunity, embryonic development, carcinogenesis and cell-death. This information is contained and utilized through highly specific binding interactions mediated by the detailed three dimensional-topological form of the specific carbohydrate. It is of great value to be able to arrange and to connect these moities in various arrays in a controlled manner. This may be

done either by connecting carbohydrate recognition groups along an oligomeric backbone, as done by for random vinyl copolymers containing functionalized sialic acid groups, which were shown to inhibit hemagluttinin binding (J. Am. Chem. Soc., 113, 686, 1991) or by arranging multiple carbohydrate groups with appropriate spacers on a suitable structural scaffold so that the carbohydrate groups are oriented in space in such a way that they can bind selectively to the target (cf., eg., J. Am. Chem. Soc., 113, 5865, 1991; ibid., 5865). Oxazolone-derived carbohydrate mimetics may be synthesized from carbohydrate modules containing functional groups, such as carboxylic acid halides, carboxylic acids, alcohols, thiols, amines, aldehydes, ketones, together with any other groups which are compatible with the oxazolone-forming and catenating reactions outlined above, thus allowing the carbohydrates to be attached to a basic scaffold, or to be arrayed along a backbone in a precise controlled manner. Examples for the synthesis of such carbohydrate modules are outlined below.

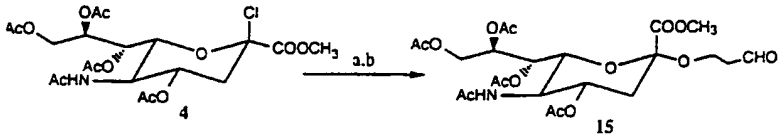
Module 1.



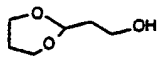
(a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -60 °C

(b) Ac_2O, Pyridine, CH_2Cl_2, rt

Module 2.

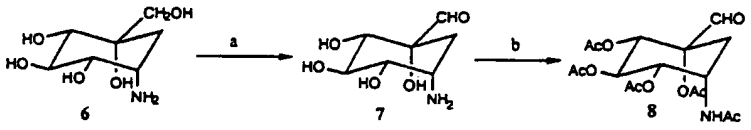


(a) 2-(2-hydroxylethyl)-1,3-dioxane, Ag-Salicylate, THF, rt
(b) aq HCl, THF, rt.



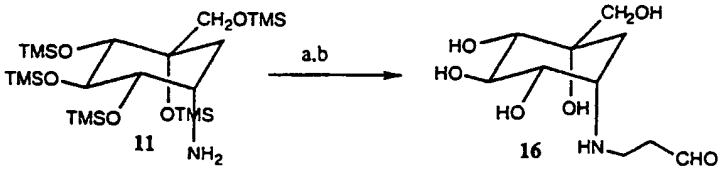
2-(2-hydroxylethyl)-1,3-dioxane

Module 3.

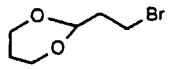


(a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -60 °C
(b) Ac_2O, Pyridine, CH_2Cl_2, rt

Module 4.



(a) 2-(2-bromoethyl)-1,3-dioxane, THF, rt
(b) HCl, THF, rt



2-(2-bromoethyl)-1,3-dioxane

4.3.4.3. Synthesis of Pharmacophore Mimetics

Background

The physical principle governing the binding of a natural ligand or substrate to a receptor or active site of an enzyme, nucleotide or carbohydrate are the same principles governing the binding of non-peptide, non-nucleotide and non-carbohydrate compounds (competitive inhibitors or agonists). The modification of a known biologically active compound as a lead or prototype, then synthesizing and testing its structural congers, homologues or analogues is a basic strategy for the development of new therapeutic agents. Several advantages of this method are:

- Greater probability of theses modified derivatives to possess physiological properties most similar to those of the prototype than those tested at random.
- Possibility of obtaining pharmacologically superior agents.
- Economical production of a new drug.
- Structure-activity relationships can be established to assist in further developments.

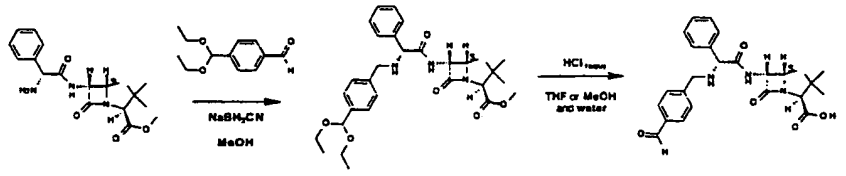
The objectives of any drug discovery program are: (a) to obtain drugs that have more desirable properties than the prototype in potency, specificity, stability, pharmalogical duration, toxicity, ease of administration and cost of production; (b) the discovery of features of the molecule which impart

pharmalogical action. The term pharmacophore is used to describe these key features that imparts this pharmalogical action.

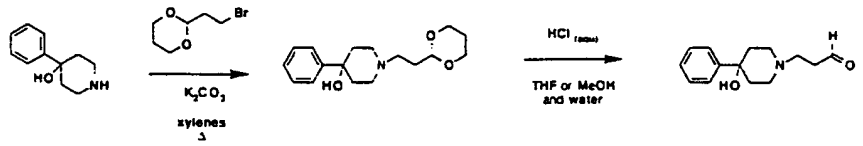
Several technologies exist where a biologically active compound, for example a protein or polypeptide, is attached to a solid support, such as a resin or glass surface. These linked compounds show diverse inhibitory activity, an indication that the ability of linked molecules to retain its binding properties despite the partial loss of mobility.

There are a wide variety of general pharmacophores known which display specific known modes of activity, e.g., ß-lactam actibacteric, interfering with bacterial cell wall; piperidine and peperizine, which can act as psychotropic agents or anticholinergics; and xanthines as stimulants. The following general schemes outline the synthesis of pharmacophore molecules, for inclusion in the various aoxazolone-derived polymer backbone forming reactions described above. Detailed examples are given below.

1. SYNTHESIS OF DA-AMINO-(*N*-(4-(OXOMETHYL)BENZYL)BENZYL-PENICILLIN:

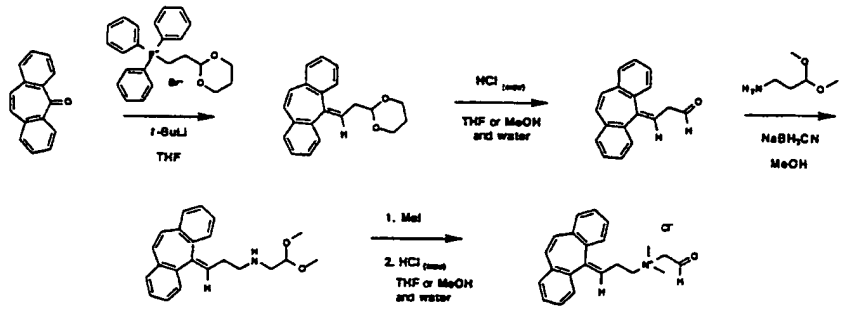


2. SYNTHESIS OF 4-HYDROXY-*N*-(2-(1,3-DIOXYL)-ETHYL)-4-PHENYLPIPERIDINE:



A solution of 4-Hydroxy-*N*-(2-(1,3-dioxyl)-ethyl)-4-phenylpiperidine (x mg, x mmol), dissolved in an appropriate solvent such as methanol / water or THF / water, with an equimolar amount of aqueous 0.5N HCl is stirred at 50 °C for 4 hours. The reaction mixture is diluted in a suitable solvent such as methylene chloride or diethyl ether, and extraceted with saturated aqueous $NaHCO_3$ to neutralize the acid, followed by brine. The solvent is removed on a rotary evaporator to afford a solid (x g, x%). A portion is recrystallized to yeild a sample for analysis.

3. SYNTHESIS OF 5*H*-5-((1,3-DIOXAN-2-YL)-2-ETHENYL)-DIBENZO[A,D]CYCLOHEPTENE:



4.4 Fabrication of Ozaxolone-Derived Macromolecular Structures Capable of Specific Molecular Recognition

In an embodiment of the invention oxazolone-derived molecular building blocks may be utilized to construct new macromolecular structures capable of recognizing specific molecules ("intelligent macromolecules"). The "intelligent macromolecules" may be represented by the following general formula:

P - C - L - R

where, R is a structure capable of molecular recognition;

L is a linker;

P is a macromolecular structure serving as a supporting platform;

C is a polymeric structure serving as a coating which surrounds P.

Structure R may be a native ligand or a biological ligand-acceptor or a mimetic thereof, such as those described above.

Linker L may be a chemical bond or one of the linker structures listed above, or a sequence of subunits such as amino acids, aminimide monomers, oxazolone-derived chains of atoms, etc.

Polymeric coating C may be attached to the supporting platform either via covalent bonds or "shrink wrapping," i.e. the bonding that results when a surface is subjected to coating polymerization is well known to those skilled in the art. This coating element may be

1) a thin crosslinked polymeric film 10 - 50 Angstroms in thickness;

2) a crosslinked polymeric layer having controlled microporosity and variable thickness, or

3) a controlled microporosity gel. When the support platform is a microporous particle or a membrane, as described below, the controlled microporosity gel may be engineered to completely fill the porous structure of the support platform. The polymeric coatings may be constructed in a controlled way by carefully controlling a variety of reaction parameters such as the nature and degree of coating crosslinking, polymerization initiator, solvent, concentration of reactants, and other reaction conditions, such as temperature,

agitation, etc., in a manner that is well known to those skilled in the art.

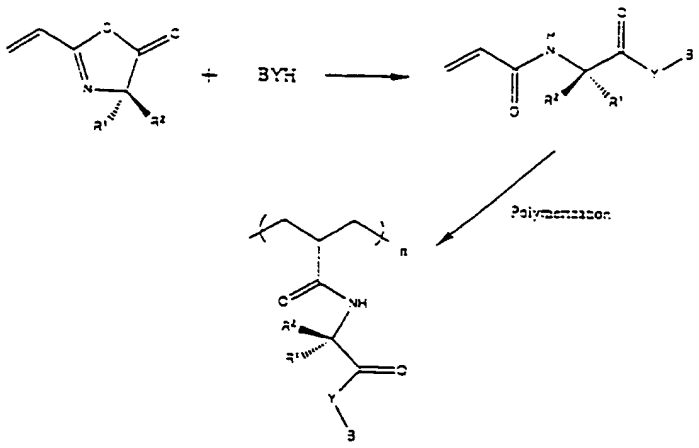
The support platform P may be a pellicular material having a diameter (dp) from 100 Angstroms to 1000 microns, a latex particle (dp 0.1 - 0.2 microns), a microporous bead (dp 1 - 1000 microns), a porous membrane, a gel, a fiber, or a continuous macroscopic surface. These may be commercially available polymeric materials, such as silica, polystyrene, polyacrylates, polysulfones, agarose, cellulose, etc. or synthetic oxazolone-containing polymers such as those described below.

Any of the elements P, C, L, or R containing an oxazolone-derived structure is derived from a form of the element containing a precursor to the oxazolone-derived structure. The multisubunit recognition agents above are expected to be very useful in the development of targeted therapeutics, drug delivery systems, adjuvants, diagnostics, chiral selectors, separation systems, and tailored catalysts.

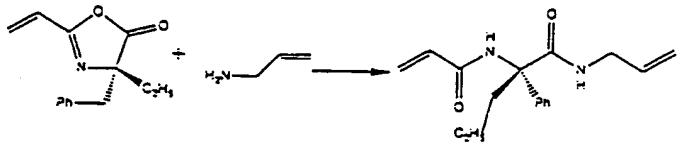
In the present specification the terms "surface", "substrate", and "structure" refer to either P, P linked to C or P linked to C and L as defined above.

4.5 Chiral Alkenyl Azlactone Monomers and Polymerization Products

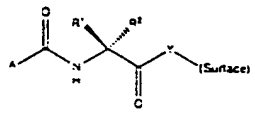
When used on an alkenyl azlactone, the azlactone ring-opening addition reaction discussed above may be used to directly produce a wide variety of chiral vinyl monomers. These may be polymerized or copolymerized to produce chiral oligomers or polymers, and may be further crosslinked to produce chiral beads, membranes, gels, coatings or composites of these materials.

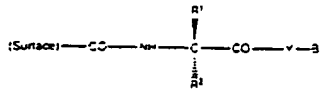


Other useful monomers, which may be used to produce chiral crosslinkable polymers, may be produced by nucleophilic opening of a chiral 2-vinyl oxazolone with a suitable amino alkene or other unsaturated nucleophile.



Vinyl polymerization and polymer-crosslinking techniques are well-known in the art (see, e.g., U.S. Patent No. 4,981,933) and are applicable to the above preferred processes.





4.6 Combinatorial Libraries Derived From Oxazolone Modules

The synthetic transformations of oxazolones outlined above may be readily carried out on solid supports in a manner analogous to performing solid phase peptide synthesis, as described by Merrifield and others (see for example, Barany, G., Merrifield, R.B., Solid Phase Peptide Synthesis, in The Peptides Vol. 2, Gross E., Meienhofer, J. eds., p. 1-284, Acad. Press, New York 1980; Stewart, J.M., Yang, J.D., Solid Phase Peptide Synthesis, 2nd ed., Pierce Chemical Co., Rockford, Illinois 1984; Atherton, E., Sheppard, R.C., Solid Phase Peptide Synthesis, D. Rickwood & B.D. Hames eds., IRL Press ed. Oxford U. Press, 1989). Since the assembly of the oxazolonederived structures is modular, *i.e.*, the result of serial combination of molecular subunits, huge combinatorial libraries of oxazolone-derived oligomeric structures may be readily prepared using suitable solid-phase chemical synthesis techniques, such as those of described by Lam (K.S. Lam, et al. Nature 354, 82 (1991)) and Zuckermann (R.N. Zuckermann, et al. Proc. Natl. Acad. Ser. USA, 89, 4505 (1992); J.M. Kerr, et al., J. Am Chem. Soc. 115, 2529 (1993)). Screening of these libraries of compounds for interesting biological activities, *e.g.*, binding with a receptor or interacting with enzymes, may be carried out using a variety of approaches well known in the art. With "solid phase" libraries (*i.e.*, libraries in which the ligand-candidates remain attached to the solid support particles used for their synthesis) the bead-staining technique of Lam may be used. The technique involves tagging the ligand-candidate acceptor, *e.g.*, an enzyme or cellular receptor of interest, with an enzyme (*e.g.*, alkaline

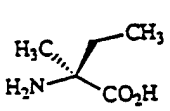
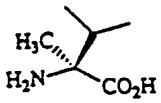
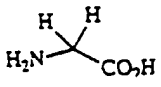
phosphatase) whose activity can give rise to color prodution thus staining library support particles which contain active ligands-candidates and leaving support particles containing inactive ligand-candidates colorless. Stained support particles are physically removed from the library (*e.g.*, using tiny forceps tht are coupled to a micromanipulator with the aid of a microscope) and used to structurally identify the biologically active ligand in the library after removel of the ligand acceptor from the complex by *e.g.*, washing with 8M guanidine hydrochloride. With "solution-phase" libraries, the affinity selection techniques described by Zuckermann above may be employed.

An especially preferred type of combinatorial library is the encoded combinatorial library, which involves the synthesis of a unique chemical code (*e.g.*, an oligonucleotide or peptide), that is readily decipherable (*e.g.*, by sequencing using traditional analytical methods), in parallel with the synthesis of the ligandcandidates of the library. The structure of the code is fully descriptive of the structure of the ligand and used to structurally characterize biologically active ligands whose structures are difficult or impossible to elucidate using traditional analytical methods. Coding schemes for construction of combinatorial libraries have been described recently (for example, see S. Brenner and R.A. Lerner, Proc. Natl. Acad. Sci. USA 89, 5381 (1992); J.M. Kerr, et al. J. Am. Chem. Soc. 115, 2529 (1993)). These and other related schemes are contemplated for use in constructing encoded combinatorial libraries of oligomers and other complex structures derived from oxazolones.

The power of combinatorial chemistry in generating screenable libraries of chemical compounds *e.g.*, in connection with drug discovery, has been described in several publications, including those mentioned above. For example, using the "split solid phase synthesis" approach outlined by Lam et al. the random incorporation of 20 oxazolones into pentameric structures, wherein each of the

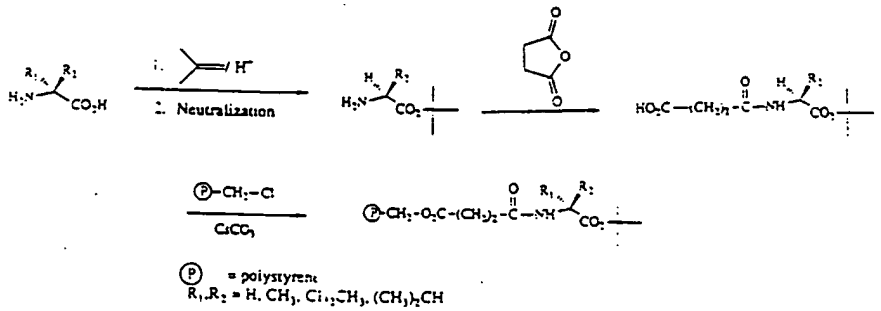
five subunits in the pentamer is derived from one of the oxazolones, produces a library of 20^5 = 3,200,000 peptidomimetic ligandcandidates, each ligand-candidate is attached to one or more solid-phase synthesis support particles and each such particle contains a single ligand-canditate type. This library can be constructed and screened for biological activity in just a few days. Such is the power of combinatorial chemistry using oxazolone modules to construct new molecular candidates.

The following is one of the many methods that are being contemplated for use in constructing random combinatorial libraries of oxazolone-derived compounds; the random incorporation of three oxazolones derived from the amino acids glycine methyl-ethyl-glycine ,and isopropyl methyl glycine to produce 27 trimeric structures linked to the support via a succinoyl linker is given as an example.

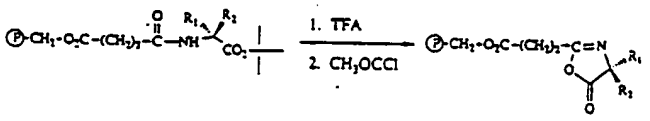
(1) A suitable solid phase synthesis support, *e.g.*, the chloromethyl resin of Merrifield, is split into three equal portions.

(2) Each portion is coupled to one and one of the glycines shown above after conversion to the acylated *t*-butyl ester derivative:



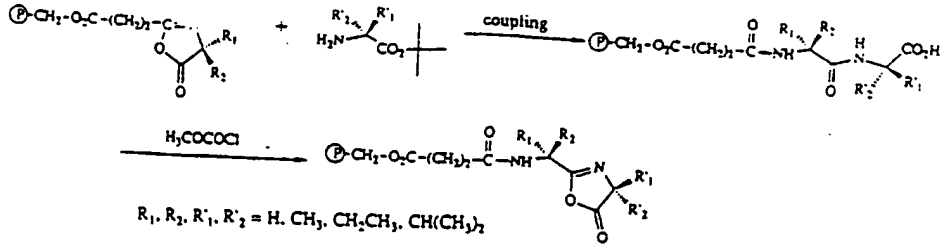
The conditions for carrying out the above transformations are well known and used routinely in the art of peptide synthesis as described in the references given above.

(3) Each amino acyl resin portion is treated with an acid solution such as neat trifluoroacetic acid (TFA), or preferably, a 1:1 mixture of TFA and CH_2Cl_2, to remove the *t*-Bu blocking group. The resulting acyl amino acid resin is treated with ethyl chloroformate as described above producing the oxazolone resin.



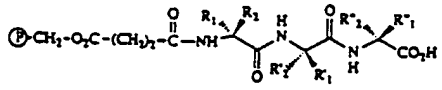
(4) The three oxazolone resin portions are thoroughly mixed and the resulting mixture is split into three equal portions.

(5) Each of the resin portions is coupled to a different glycine protected as *t*-butyl ester using the conditions described above; the amide product is deprotected as described above, for each of the resin portions and cyclized to the oxazolone using the reaction with ethyl chloroformate.



(6) The resulting resin portions are mixed thoroughly and then split again into three equal portions.

(7) Each of the resin portions is coupled to a different glycine, containing a carboxyl protected as the *t*-butyl ester, and the product is deprotected using TFA as described above; the resin portions are mixed producing a library containing 27 types of resin beads, each type containing a single oxazolonederived tripeptide analog linked to the support via a succinoyl linker; this linker may be severed using acidolysis to produce a "solution-phase" library of peptides, whose N-terminus is succinoylated.

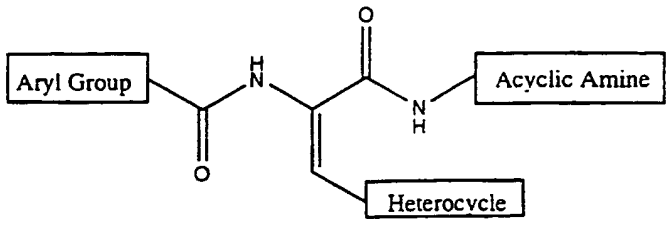


Many modifications of this general scheme are envisioned, including the direct attachment of the ligand candidates via a C-N bond using a benzhydryl support, which would allow the straight forward detachment of the ligand candidates from the support via acidolysis for further study ("one-head, one-peptide-analog synthesis").

The ability of these chemistries to be modularly connected in a structure directed manner gives us a unique ability to produce directed combinatorial libraries by systematically varying structural elements around a structural motif. This is exemplifyed for the generation of a matrix of 16

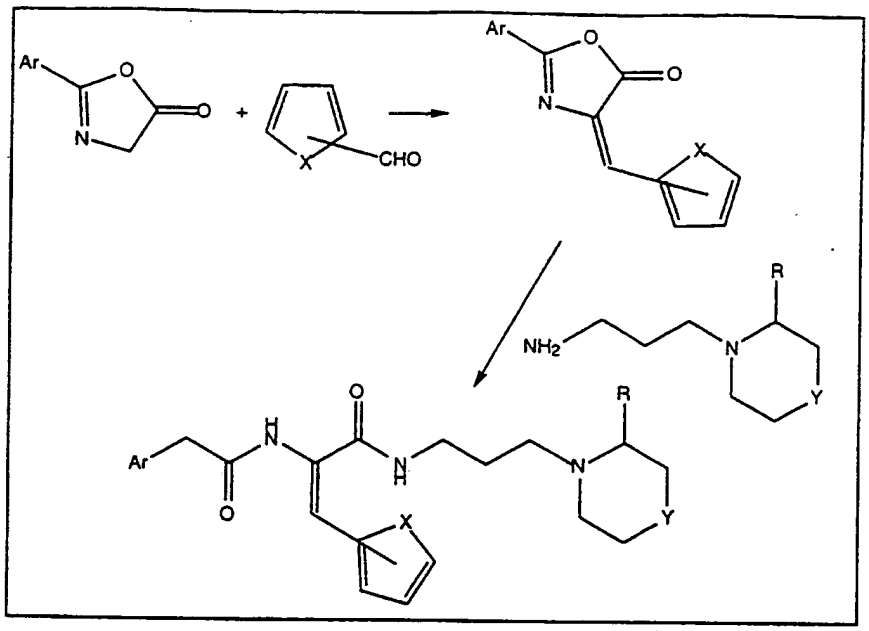
molecules around the following aryl-heterocycle-alicyclic amine structural theme.

Theme:



This was done by reacting the 2-phenyl and 2-(2-naphthyl)-5-oxazolones (produced by reacting the lithium salt of glucine with the aryl acid chlorides, followed by cyclization with ethyl chloroformate at 0 C) with 2-furfural, 3-fufural, 2-thiophenal and 3-thiophenyl to produce oxazolones functionalized at the 4-position, followed by ring-opening addition of 4-(3-aminopropylmorpholine and 1-(3-aminopropyl)-2-pipicoline to form the adducts shown. this was accomplished by carrying out the reactions in individual vials such that each vial contained one pure final compound as follows:

1.) equimolar quantities of the oxazolone and the aldehyde dissolved in dry benzene (25ml/gm reactants) were heated to 75 C for 15 minutes; 2.) the reaction mixture was cooled to 10 C and the amine was added dropwise with stirring; 3.) the mixture was re-heated to 75 C for 20 minutes and 4.) the solvent was removed in vacuo to give the crude solid product.



Ar	X / Isomer	R / Y
Ph	O 2-	H O
Ph	S 2-	H O
Ph	O 2-	CH3 CH2
Ph	S 2-	CH3 CH2
Naphthyl	O 2-	H O
Naphthyl	S 2-	H O
Naphthyl	O 2-	CH3 CH2
Naphthyl	S 2-	CH3 CH2
Ph	O 2-	H O
Ph	S 2-	H O
Ph	O 2-	CH3 CH2
Ph	S 2-	CH3 CH2
Naphthyl	O 2-	H O
Naphthyl	S 2-	H O
Naphthyl	O 2-	CH3 CH2
Naphthyl	S 2-	CH3 CH2

4.7 Design and Synthesis of Oxazolone-Derived Glycopeptide Mimetics

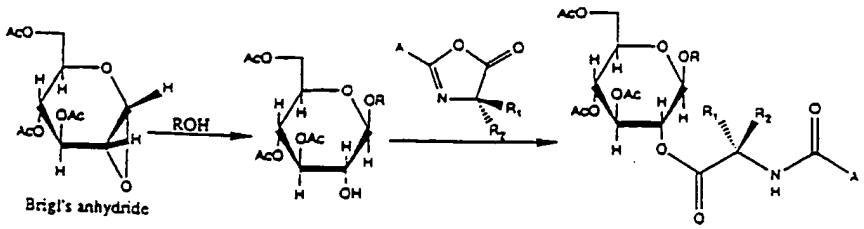
A great variety of saccharide and polysaccharide structural motifs incorporating oxazolone-derived structures are contemplated including but not limited to the following.

(1) Oxazolone-derived structures which mimic native peptide ligands capable of binding to saccharide and polysaccharide receptors using the design and synthesis techniques that are described above.

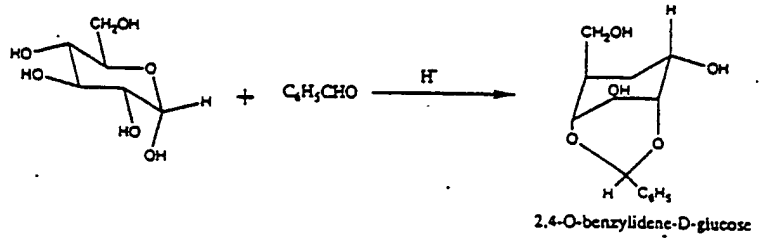
(2) Oxazolone-derived structures linking mono-, oligo- or polymeric saccharides with each other or with other structures capable of recognizing a ligand acceptor.

A wealth of chemical methods for synthesis of the above saccharides are available. The art of carbohydrate chemistry describes numerous sugars of variety of sizes with selectively blocked functional groups, which allows for selective reactions with oxazolone and related species producing the desired products (see Comprehensive Organic Chemistry, Sir Derek Barton, Chairman of Editorial Board, Vol. 5, E. Haslam, Ed., pp. 687-815; A. Streitwieser, C.H. Heathcock, E. Kosower, Introduction to Organic Chemistry, 4th Edition, MacMillan Publ. Co., New York, pp. 903-949.

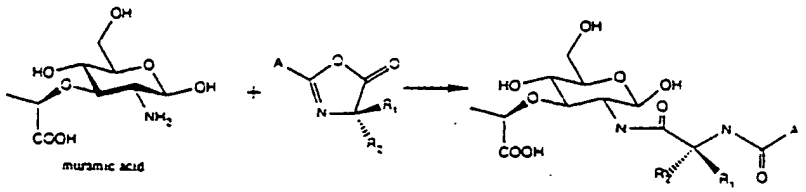
For example, Brigl's anhydride shown below can be reacted with unhindered alcohols to produce ßglucosides using well-known experimental conditions. The resulting sugar, blocked at all positions except position 2, can be used acid catalyst such as BF_3 in a suitable inert organic solvent to open a suitable oxazolone using the reaction conditions described above, *e.g.*, in the absence or presence of a Lewis (*e.g.*, EtOAC, dioxane, etc.).



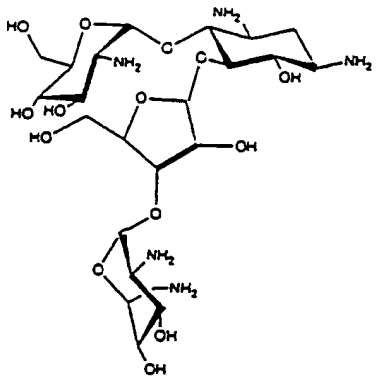
Similarly the sugar that results from reaction of D-glucose with benzaldehyde can be readily blocked at positions 1 and 6, by sequential reactions with an alcohol in the presence of acid, and tritylation using techniques well known in the art of carbohydrate chemistry. The resulting sugar, with position 3 unblocked can be used selectively as described above to derivatize a desired oxazolone structure.



A suitable oxazolone can also be ring-opened by a sugar containing reactive amino substituents, *i.e.*, an aminosaccharide or polyaminosaccharide. For example, reaction with muramic acid is expected to proceed as follows.



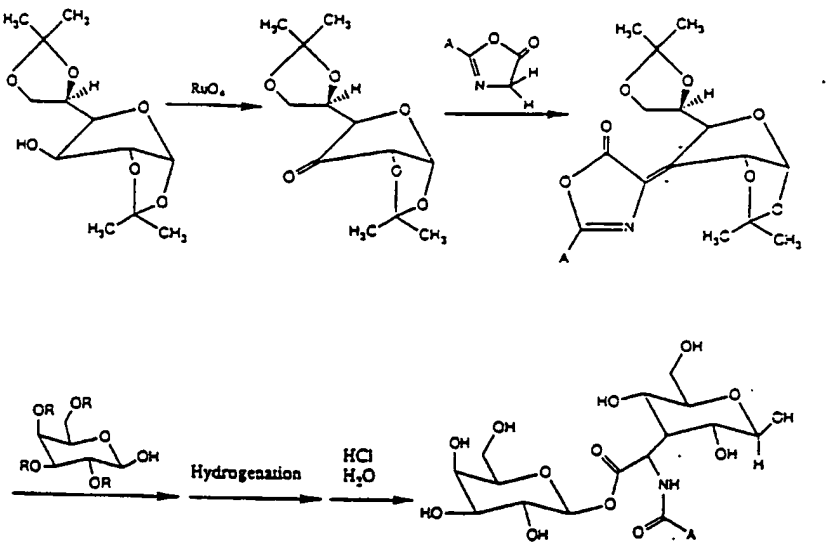
Similar treatment which is shown below, of the structurally interesting ambecide paromomycin, with 1 to 5 equivalents of a tailored oxazolone is expected to produce a series of novel structures in which a branched tetrasaccharide scaffold supports peptidomimetic structures derived from oxazolones in a geometrically defined manner.



Paromomycin

(3) Use of oxazolone-derived structures as replacements of glycosidic linkages.

Selective blocking of all but one hydroxyl in a sugar allows the selective oxidation of the hydroxyl to the carbonyl-derivative, which can then be used in an aldol-type condensation reaction with a methylene oxazolone to produce an alkene oxazolone; this can then be ring-opened. by *e.g.*, the anomeric hydroxyl of a sugar to give a novel saccharide after deprotection.



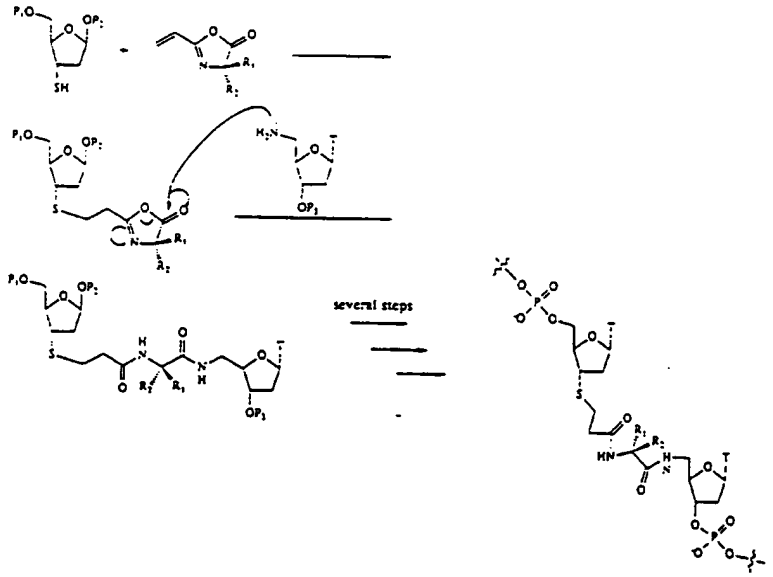
4.8 Design and Synthesis of Oxazolone-Derived Oligonucleotide Mimetics

The art of nucleotide and oligonucleotide synthesis has provided a great variety of suitably blocked and activated furanoses and other intermediates which are expected to be very useful in the construction of oxazolonederived mimetics (Comprehensive Organic Chemistry, Sir Derek Barton, Chairman of Editorial Board, Vol. 5, E. Haslam, Editor, pp. 23-176).

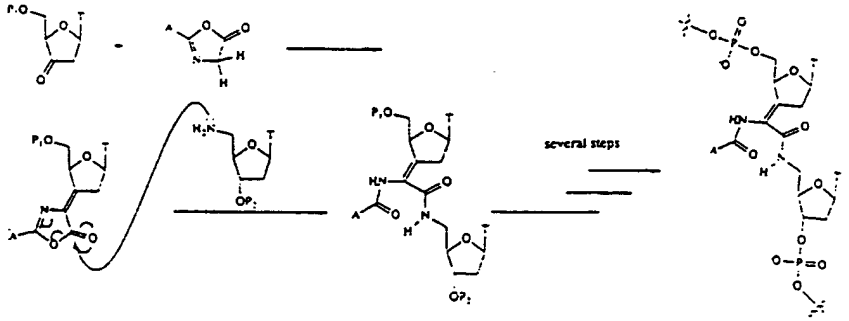
A great variety of nucleotide and oligonucleotide structural motifs incorporating oxazolonederived structures are contemplated including, but not limited to, the following.

(1) For the synthesis of oligonucleotides containing peptidic oxazolone-derived linkers in place of the phosphate diester groupings found in native

oligonucleotides, the following approach is one of many that is expected to be useful.



(2) For the synthesis of structures in which an oxazolone-derived grouping is used to link complex oligonucleotidederived units, an approach such as the following is expected to be useful.



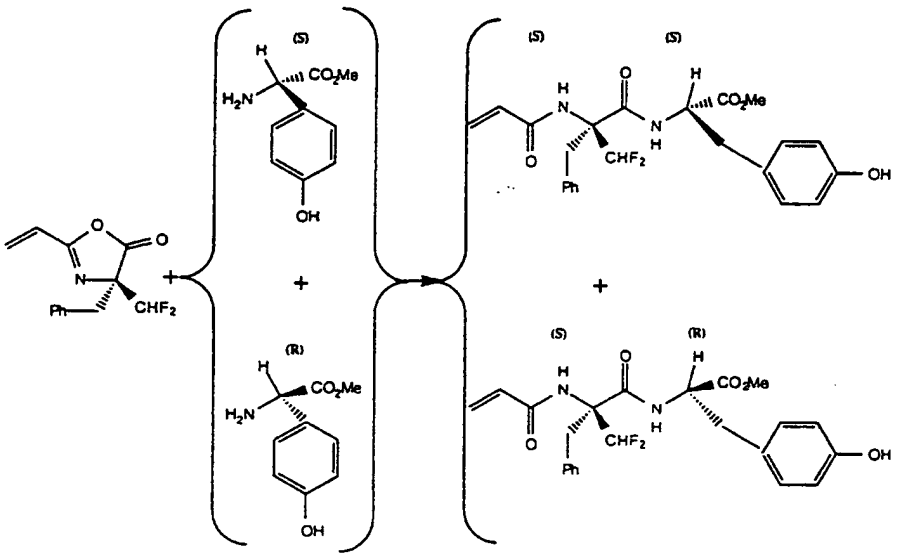
EXAMPLES

In order to exemplify the results achieved using the oxazolone derivatives of the present invention, the following examples are provided without any attempt to limit the scope of the instant invention to the discussion therein, all parts and percentages are by weight, unless otherwise indicated.

EXAMPLE 1.

CHARACTERIZATION OF THE ENANTIOMETRIC PURITY OF OXFENACINE

This example teaches the use of the ring opening reaction of the pure chiral isomer azalactone (S)-(-)-4difluoromethyl-4-benzyl-2-vinyl-5-oxazolone (1) with racemic mixtures of the methyl esters of (R)- and (S)-phydroxyphenylglycine to form the diastereomeric conjugates (2) and (3), as shown:



These diastereomers can be separated by standard HPLC methods on normal-phase silica to quantitatively assay the enantiomeric composition of the starting phydroxyphenylglycines from which the esters are produced.

The (S)-isomer of p-hydroxyphenylglycine (oxfenacine) is an effective therapeutic agent for promoting the oxidation of carbohydrates when this process is depressed by high fatty acid utilization levels (such as occurs in ischemic heart disease), and is also an important chiral intermediate in the production of penicillin, amoxicillin and several other semisynthetic antibiotics, including the cephalosporins. Oxfenacine is prone to racemization, and the assay for chiral purity described in this example therefore represents a useful development and quality-control tool.

RESOLUTION OF RACEMIC *P*-HYDROXYPHENYL GLYCINE ESTERIFICATION OF P-HYDROXYPHENYL GLYCINE

0.3 g (0.2 ml) thionyl chloride was added dropwise to 5 ml of a stirred solution of 0.4 g of the stereoisomeric mixture of 4-hydroxyphenylglycine enantiomers to be characterized in methanol and the temperature of the mixture kept between 10 and 20^{O}C with ice cooling. The reaction was allowed to proceed at room temperature for 1 hour. The methanol was then removed at room temperature under aspirator vacuum (10 torr) on a rotary evaporator and a solid was obtained. This solid was dissolved in 10 ml of deionized water and the pH adjusted to 9.2 with 0.88 M ammonium hydroxide. The solution was then stirred for 1 hour at 10^{O}C and the precipitated solid ester mixture was filtered off, washed with deionized water and dried at 45^{O}C under vacuum to give 0.41 g of product (94%).

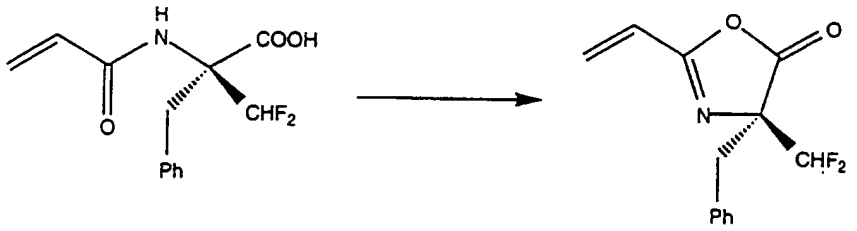
RING-OPENING ADDITION.

0.181 g (0.001 mol) of the esterified 4-hydroxyphenylglycine prepared as outlined above was dissolved in 10 ml of peroxide-free dry dioxane. To this mixture was added 0.251 g (0.001 mol) of (S)-4difluoromethyl-4-benzyl-2-vinyl-5-oxazolone, and the resulting solution heated at reflux for 2 hours. The dioxane was removed by rotary evaporation and 0.43 g (100%) of the pale yellow solid amide residue was isolated.

HPLC ANALYSIS.

A solution of the diastereomeric amides was prepared in methylene chloride at a concentration of 7 mg/ml. This solution was injected into a DuPont Model 830 liquid chromatograph equipped with a detector set at 254 nm using a 20 ul loop valve injection system. The sample was chromatographed on a 25 cm x 0.4 cm stainless steel HPLC column packed with 5_ Spherisorb S5W silica gel using a 98/1/1 cyclohexane/n-butanol/isopropanol mobile phase at a flow rate of 0.9 ml/min. The enantiomeric amide conjugates were then quantitated using a calibration curve generated with a series of synthetic mixtures containing varying ratios of the two pure enantiomers. The pure Lisomer was purchased from Schweizerhall Inc. The pure Disomer was prepared from the commercially available D,Lracemate obtained from MTM Research Chemicals/Lancaster Synthesis Inc. by the method of Clark, Phillips and Steer (J. Chem. Soc., Perkins Trans. I at 475 [1976]).

(S)-4-DIFLUOROMETHYL, 4-BENZYL-2-VINYL-5-OXAZOLONE



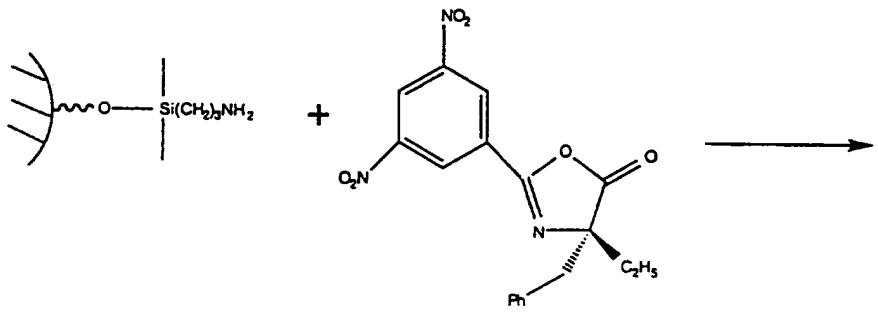
5.43 g (0.05 mol) of ethyl chloroformate was added with stirring to 13.46 g (0.05 mol) of N-acryloyl(S)-2-difluoromethyl phenylalanine in 75 ml of dry acetone at room temperature. 7.0 ml (0.05 mol) of triethylamine were then added dropwise over a period of 10 min., and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The triethylamine hydrochloride was removed by filtration, the cake was slurried in 25 ml of acetone and refiltered. The combined filtrates were concentrated to 50 ml on a rotary evaporator, refiltered, cooled to -30°C and the crystallized product was collected by filtration and dried *in vacuo* to give 10.05 g (80%) of (S)-4-difluoromethyl-4benzyl-2-vinyl azlactone. NMR ($CDCl_3$); CH_2 = CH - chemical shifts, splitting pattern in 6 ppm region and integration ratios diagnostic for structure. FTIR (mull) strong azlactone CO band at 1820 cm^{-1}.

SYNTHESIS OF N-ACRYLOYL-(S)-2-DIFLUOROMETHYL PHENYLALANINE.

21.5 g (0.1 mol) (S)-2-difluoromethyl phenylalanine, prepared using the method described by Kolb and Barth (Liebigs Ann. Chem. 1668 (1983)), was added with stirring to a solution of 8.0 g (0.2 mol) of sodium hydroxide in 100 ml water and stirred at this temperature until complete solubilization was achieved. 9.05 g (0.1 mol) acryloyl chloride was then added dropwise with stirring, keeping the temperature at 10-15°C with external cooling. After addition was complete, stirring was

continued for 30 min. To this solution 10.3 ml (0.125 mol) of concentrated hydrochloric acid was added over a 10-min. period, keeping the temperature at 15°C. After addition was complete, the reaction mixture was stirred an additional 30 min., cooled to 0°C, and the solid product was collected by filtration, washed well with ice water and pressed firmly with a rubber dam. The resulting wet cake was recrystallized from ethanol/water to yield 18.8 g (70%) of N-acryloyl-(S)-2-difluoromethyl phenylalanine. NMR ($CDCl_3$): chemical shifts, CH_2 = CH - splitting pattern and integration ratios diagnostic for structure

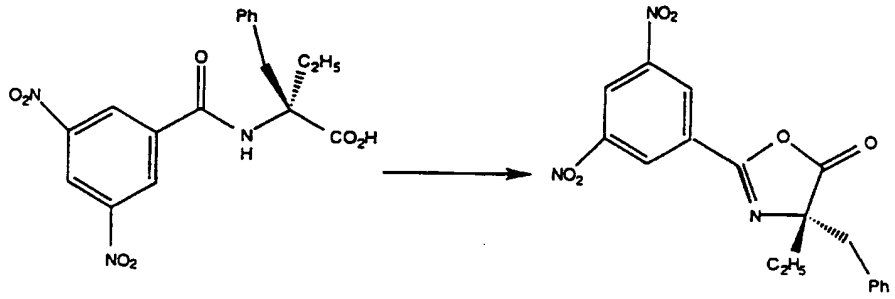
EXAMPLE 2.
PREPARATION OF CHIRAL CHROMATOGRAPHIC STATIONARY PHASE RING OPENING FORMATION OF CONJUGATE WITH AMINOPROPYL SILICA



5.0 g of aminopropyl-functionalized silica was slurried in 100 ml benzene in a three-necked flask equipped with a stirrer, a heating bath, a reflux condenser and a Dean-Stark trap. The mixture heated to reflux and the water removed azeotropically. 3.69 g (0.01 mol) of (S)-

4-ethyl,4-benzyl-2-(3',5'-dinitrophenyl)-5oxazolone was added and the mixture was heated at reflux for 3 hours. The mixture was subsequently cooled, and the silica collected on a Buechner filter and washed with 50 ml benzene. The wet cake was reslurried in 100 ml methanol and refiltered a total of four times. The resulting product was dried in a vacuum oven set for 30" and 60°C to yield 4.87 g functionalized silica. The bonded phase was packed into a 25 cm x 0.46 cm stainlesssteel HPLC column from methanol, and successfully used to separate a series of mandelic acid derivatives using standard conditions.

SYNTHESIS OF (S)-4-ETHYL,4-BENZYL-2-(3',5'-DINITROPHENYL)-5-OXAZOLONE



1.09 g (0.01 mol) of ethyl chloroformate was added with stirring to 3.87 g (0.01 mol) N-3,5-dinitrobenzoyl(S)-2-ethyl phenylalanine in 75 ml dry acetone at room temperature. 1.4 ml (0.01 mol) of triethylamine was added dropwise over a 10-min. period and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The triethylamine hydrochloride was removed by filtration and the cake was slurried with 25 ml acetone and refiltered. The combined filtrates were concentrated to 50 ml on a rotary evaporator, refiltered, cooled to 30°C and the crystallized roduct was collected by filtration and dried *in vacuo* to yield 2.88 g (78%) of (S)-4-ethyl-4-benzyl-2-(3',5'-

dinitrophenyl)azlactone. NMR ($CDCl_3$): Frequencies and integration ratios diagnostic for structure. FTIR: strong azlactone band at ca. 1820 cm^{-1}.

N-3,5-DINITROBENZLOYL-(S)-2-ETHYLPHENYLALANINE

19.3 g (0.1 mol) of (S)-2-ethylphenylalanine, prepared from (S)-phenylalanine and ethyl iodide using the method described by Zydowsky, de Lara and Spanton (55 J. Org. Chem. 5437 (1990)) was added with stirring to a solution of 8 g (0.2 mol) sodium hydroxide in 100 ml water and cooled to about 10°C. The mixture was then stirred at this temperature until complete solubilization was achieved. 23.1 g (0.1 mol) 3,5-dinitrobenzoyl chloride was then added dropwise with stirring, keeping the temperature at 10-15°C with external cooling. After this addition was complete, stirring was continued for 30 min. To this solution was added 10.3 ml (1.25 mol) of concentrated HCl over a 10 min. period, again keeping the temperature at 15°C. During this addition a white solid formed. After the addition was complete, the reaction mixture was stirred for an additional 30 min., cooled to 0_C and the white solid was collected by filtration, washed well with ice water and pressed firmly with a rubber dam. The resulting wet cake was recrystallized from ethanol/water and dried in a vacuum oven set for 30" at 60°C to yield 27.1 g (70%) N-3,5-dinitrobenzoyl-(S)-2ethyl phenylalanine.

EXAMPLE 3.

SYNTHESIS OF PREPARATION OF AMINOPROPYL-FUNCTIONALIZED SILICA.

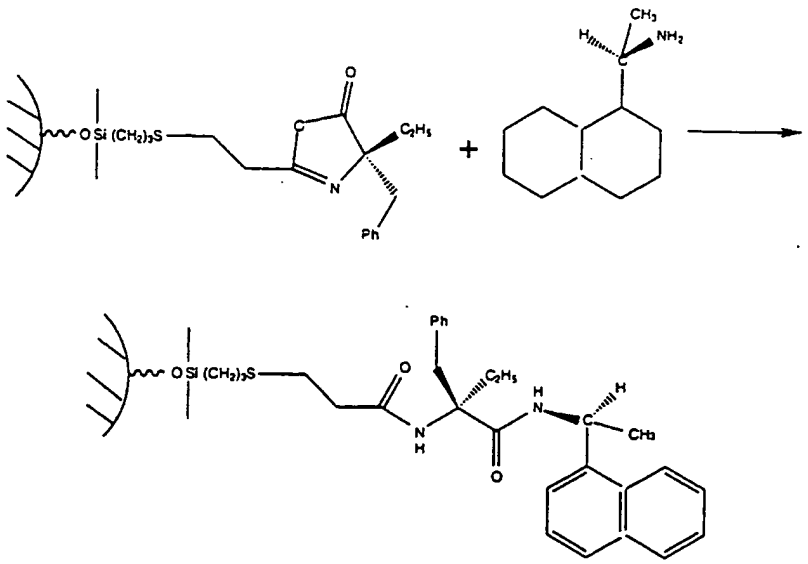
200 g 015M Spherosil (IBF Corporation) was added to 500 ml toluene in a one-liter three-necked roundbottomed flask equipped with a Teflon paddle stirrer, a thermometer and a vertical condenser set up with a DeanStark trap through a claisen adaptor. The slurry was stirred, heated to a bath temperature of 140°C and the

water azeotropically removed by distillation and collected in the Dean-Stark trap. The loss in toluene volume was measured and compensated for by the addition of incremental dry toluene. 125.0 g of 3-aminopropyl trimethoxysilane was added carefully through a funnel and the mixture stirred and refluxed for 3 hours with the bath temperature set at 140°C. The reaction mixture was cooled to about 40°C and the resulting functionalized silica collected on a Buechner filter. The silica was then washed twice with 50 ml toluene, sucked dry, reslurried in 250 ml toluene, refiltered, reslurried in 250 ml methanol and refiltered a total of three times. The resulting methanol wet cake was dried in a vacuum oven set for 30" at 60°C to yield 196.4 g aminopropyl silica.

EXAMPLE 4

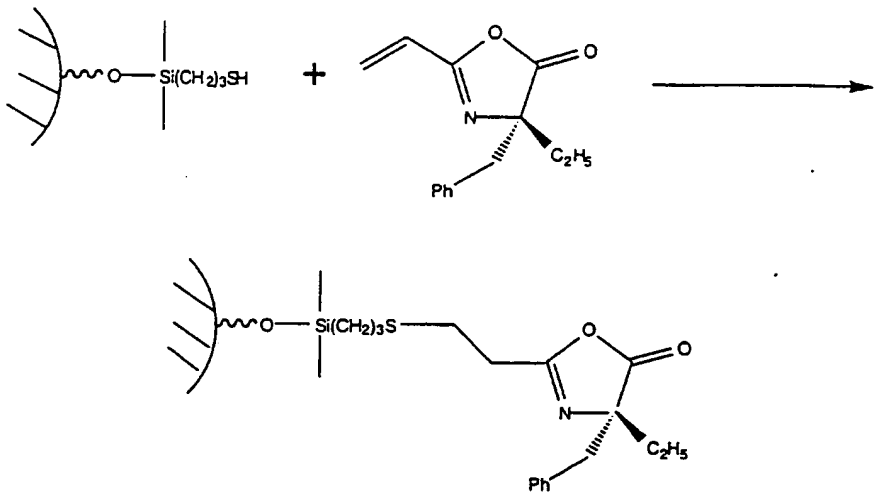
RING-OPENING CONJUGATION OF (S)-1-(1NAPHTHYL)ETHYLAMINE WITH THE MICHAEL-ADDITION PRODUCT OF AMINOMERCAPTO-FUNCTIONALIZED SILICA AND (S)-4ETHYL-4-BENZYL-2-ACRYLOYL-5-OXAZOLONE TO PRODUCE A CHIRAL CHROMATOGRAPHIC STATIONARY PHASE

FORMATION OF CONJUGATE WITH (S)-(1)(1NAPHTHYL)ETHYLAMINE



10.0 g (S)-4-ethyl-4-benzyl-2-(ethylthiopropyl silica)-5-oxazolone was slurried in 100 ml benzene in a three-necked flask equipped with a stirrer, a heating bath, a reflux condenser and a Dean-Stark trap. The mixture was heated to reflux and the water was removed azeotropically. 3.42 g (0.02 mol) (S)-(-)- (1naphthyl)ethylamine was added and the mixture was heated at reflux for 6 hours. The mixture was then cooled, the silica collected on a Buechner filter and washed with 100 ml benzene. The wet cake was reslurried in 100 ml methanol and refiltered a total of four times. The product was dried in a vacuum oven set for 30" and 60^{0}C to give 9.72 g functionalized silica. The bonded phase was packed into a 25 cm x 0.46 cm stainless-steel HPLC column from methanol and successfully used to separate a series of pi-acceptor amine derivatives using standard conditions described in the Chromatography Catalog distributed by Regis Chemical, Morton Grove, Ill. 60053 (e.g., the 3,5dinitro benzoyl derivatives of racemic 2-amino-1-butanol + alpha methyl benzyl amine).

MICHAEL ADDITION BY MERCAPTOPROPYL SILICA



20 g mercaptopropyl silica was added to 200 ml benzene in a 500 ml three-necked round-bottomed flask equipped with a Teflon paddle stirrer, a thermometer and a vertical condenser set up with a Dean-Stark trap through a claisen adaptor. The slurry was stirred, heated to a bath temperature of 140^{0}C and the water azeotropically removed by distillation and collected in the Dean-Stark trap. The loss in benzene volume was measured and compensated for by the addition of incremental dry benzene. 6.88 g (0.03 mol) of (S)-4-ethyl,4-benzyl-2-vinyl-5-oxazolone was added and the mixture was stirred and refluxed for 16 hours. The reaction mixture was then cooled to about 40^{0}C. The resulting functionalized silica was collected on a Buechner filter, washed with 50 ml benzene, sucked dry, reslurried in 100 ml of methanol and refiltered a total of four time. The resulting methanol wet cake was dried in a vacuum oven set for 30" at 60^{0}C to yield 19.45 g oxazolone-functionalized silica.

SYNTHESIS OF (S)-4-ETHYL-4'-BENZYL-2-ACRYLOYL-5-OXAZOLONE.

10.9 g (0.1 mol) of ethyl chloroformate was added with stirring to 24.7 g (0.1 mol) of N-acryloyl-(S)2-ethyl phenylalanine in 250 ml dry acetone at room temperature. 14 ml (0.1 mol) of triethylamine was added dropwise over a 10-min. period and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The triethylamine hydrochloride was removed by filtration and the cake was slurried with 50 ml of acetone and refiltered. The combined filtrates were concentrated to 150 ml on a rotary evaporator, refiltered, cooled to 30^0C and the crystallized product was collected by filtration and dried *in vacuo* to yield 19.5 g (85%) (S)-4ethyl-4-benzyl-2-vinyl-5-azlactone. NMR 9CDCl): chemical shifts, CH_2 = CH - splitting pattern in 6 ppm region + integration ratios diagnostic for structure. FTIR + (mull): strong azlactone CO band in 1820 cm^{-1} region.

PREPARATION OF MERCAPTOPROPYL-FUNCTIONALIZED SILICA

200 g of 10u (80A) Exsil silica (Exmere Ltd.) was added to 500 ml toluene in a one-liter three-necked round-bottomed flask equipped with a Teflon paddle stirrer, a thermometer and a vertical condenser set up with a Dean-Stark trap through a claisen adaptor. The slurry was stirred, heated to a bath temperature of 140^0C and the water was azeotropically removed by distillation and collected in the Dean-Stark trap. The loss in toluene volume was measured and compensated for by the addition of incremental dry toluene. 110.0 g of 3-mercaptopropyl trimethoxysilane was added carefully through a funnel and the mixture was stirred and refluxed for 3 hours with the bath temperature set at 140^0C. The reaction mixture was then cooled to about 40^0C. The resulting functionalized silica was collected on a

Buechner filter, washed twice with 50 ml toluene, sucked dry, reslurried in 250 ml toluene, refiltered, reslurried in 250 ml methanol and refiltered a total of three times. The resulting methanol wet cake was dried in a vacuum oven set for 30" at 60^{0}C to yield 196.4 g of mercaptopropyl silica.

EXAMPLE 5.

SYNTHESIS OF A MIMETIC OF KNOWN HUMAN ELASTASE INHIBITOR

This example teaches the synthesis of a competitive inhibitor for human elastase based on the structure of known N-trifluoroacetyl dipeptide analide inhibitors - see, e.g., 107 Eur. J. Biochem. 423 (1980); 162 J. Mol. Biol. 645 (1982) and references cited therein.

SYNTHESIS OF N-TRIFLUOROACETYL-(S)-2-METHYL LEUCYL-(S)-2-ETHYLPHENYLALANYLP-ISOPROPYLANLIDE

0.135 g (0.001 mol) 4-isopropyl analine is dissolved in the minimum amount of an appropriate solvent, such as acetonitrile, and 0.384 g (0.001 mol) of 2-(Ntrifluoroacetyl-(S)-2-methyl leucyl)-(S)-4-methyl-4-benzyl5-oxazolone dissolved in the minimum amount of the same solvent is added gradually to the stirred solution with cooling. Following addition, the reaction mixture is allowed to come to room remperature and is stirred at room temperature for 36 hours. The solvent is then removed *in vacuo* to yield the solid N-trifluoroacetyl-(S)-2-methylleucyl-(S)-2-ethylphenylalanyl analide, useful as a competitive inhibitor of human elastase in essentially quantitative yield.

2-(N-TRIFLUOROACETYL-(S)-2-METHYLLEUCYL)-(S)-4-METHYL-4BENZYL-5-OXAZOLONE

4.1 g (0.01 mol) N-trifluoroacetyl-(S)-2-methylleucyl(S)-2-methylphenylalanine lithium salt is slurried in 50 ml of an appropriate solvent, such as dry benzene, in a three-necked round-bottomed flask equipped with a stirrer, heating bath, claisen head, downward condenser, thermometer and dropping funnel. The system is heated to 65^{0}C, and 1.09 g (0.01 mol) of ethyl chloroformate dissolved in 10 ml dry benzene is added over a 10-min. period. Addition is accompanied by the vigorous evolution of gas and the distillation of a benzene/ethanol azeotrope. Following the completion of the addition, heating is continued for 30 min. The heating bath is then removed and the slurry is stirred for an additional 15 min. The precipitated lithium chloride is carefully removed by filtration and the cake is triturated with benzene and refiltered. The combined filtrates are stripped using a pot temperature of 40^{0}C to yield 3.50 g (90%) of crude oxazolone. The product was purified by recrystallization from acetone at -30-C. FTIR (mull): Strong azlactone CO band in 1820 cm^{-1} region.

SYNTHESIS OF N-TRIFLUORACETYL-(S)-2-METHYLLEUCYL-(S)-2METHYLPHENYLALANINE.

2.23 g (0.01 mol) 2-trifluoroacetyl-(S)-4-methyl4-isobutyl-5-oxazolone is dissolved with stirring in the minimum amount of an appropriate solvent, such as acetonitrile, and 1.85 g (0.01 mol) of the lithium salt of (S)-2-methyl phenylalanine in the minimum amount of the same solvent is added gradually, and with cooling. This salt is obtained by treatment of (S)-2-methylphenylalanine (produced from (S)-phenylalanine and methyl iodide using the method of Zydoski et al., 55 J. Org. Chem. 5437 (1990)) with one equivalent of LiOH in an appropriate solvent, such as ethanol, followed by removal of the solvent *in vacuo*.

After addition of the lithium salt, the reaction mixture is allowed to warm to room temperature and is stirred at room temperature for 36 hours. The solvent is then removed *in vacuo* to yield the solid Ntrifluoroacetyl-(S)-2-methylleucyl-(S)-2methylphenylalanine lithium salt in nearly quantitative yield.

SYNTHESIS OF 2-TRIFLUOROACETYL-(S)-4-METHYL-4-ISOPROPYL-5-OXAZOLONE.

12.05 g (0.05 mol) of N-trifluoroacetyl-(S)-2methyl-leucine was stirred at room temperature in 100 ml dry acetone and 5.43 g (0.05 mol) ethyl chloroformate was added. 7.0 ml (0.05 mol) of triethylamine was added dropwise over a period of 10 min. and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The triethylamine hydrochloride was removed by filtration and the cake was slurried with 25 ml of acetone and refiltered. The combined filtrates were concentrated to 75 ml on a rotary evaporator, refiltered, cooled to -30^{0}C and the crystallized product was collected by filtration and dried *in vacuo* to yield 10.6 g (88%) of (S)-4-methyl-4-isobutyl-2-trifluoroacetyl-5-oxazolone. FTIR (mull): strong azlactone CO band in 1820 cm^{-1} region.

SYNTHESIS OF N-TRIFLUOROACETYL-(S)-2-METHYL-LEUCINE

14.5 g (0.1 mol) of (S)-2-methyl-leucine, prepared from D,L-leucine methyl ester hydrochloride using the method of Kolb and Barth (Liebig's Ann. Chem. at 1668 (1983)) was added with stirring to a solution of 8 g (0.2 mol) of sodium hydroxide in 20 ml water, cooled to 10^{0}C, and the mixture stirred at this temperature until complete solubilization was achieved. 13.25 g (0.1 mol) trifluoroacetyl chloride was then added dropwise with

stirring, keeping the temperature at 10°C with external cooling. After the addition was complete, stirring was continued for 30 min. To this solution was added, over a 10-min. period, 10.3 ml (0.125 mol) of concentrated hydrochloric acid, again keeping the temperature at 15°C During the addition, a white solid formed. After the addition was complete, the reaction mixture was stirred for an additional 30 min. and cooled to 0°C. The white solid was collected by filtration, washed well with ice water and pressed firmly with a rubber dam. The resulting wet cake was recrystallized from ethanol/water and dried *in vacuo* to give 17.4 g (72%) of N-trifluoroacetyl-(S)-2methyl-leucine which was used directly in the following step in the sequence (above).

EXAMPLE 6.
SYNTHESIS OF A PEPSTATIN MIMETIC

This example teaches the synthesis of an oxazolonederived mimetic of the known aspartyl protease inhibitor, pepstatin, which has the structure shown: This mimetic is useful as a competitive inhibitor for proteases inhibited by pepstatin.

N-isovaleryl-(S)-2-methylvaleryl-(3S,4S)-statyl-(S)-2methyl-alanyl-(3S,4S)-statine.

The Boc-protected lithium salt prepared as described below simultaneously converted to the acid form and deprotected by treatment with acid under standard deprotection conditions. 5.17 g (0.01 mol) of N-isovaleryl(S)-2-methy derivative added to 100 ml dry acetonitrile, stirred at room temperature and 3.17 g (0.01 mol) of the valyl-(S)-4-methyl-4-isopropyl-5-oxazolone was added with cooling. Once addition was complete, the mixture was heated to reflux and held at reflux for 1 hour. The solvent then stripped *in vacuo* to give a quantitative

yield of N-isovaleryl-(S)-2-methylvalyl-(3S,4S)-statyl(S)-2-methylalanyl-(3S,4S)-statine, useful as a pepstatinmimetic competitive inhibitor for aspartyl proteases which are inhibited by pepstatin (see, 23 J. Med. Chem. 27 (1980) and references cited therein). NMR (d_6 DMSO): chemical shifts, integrations and D_2O exchange experiments diagnostic for structure.

N-Boc-(3S,4S)-statyl-(S)-2-methylalanyl-(3S,4S)-statine lithium salt.

6.84 g (0.02 mol) of the Boc-protected oxazolone prepared below stirred in 100 ml of dry acetonitrile at room temperature and 3.62 g (0.02 mol) of the lithium salt of (3S,4S)-statine, prepared from statine using the method outlined below, was added with cooling. Once addition was complete, the mixture was heated to reflux and held at reflux for 1 hour. The solvent was then stripped *in vacuo* to give a quantitative yield of N-Boc-(3S,4S)-statyl-(S)2-methylalanyl-(3S,4S)-statine lithium salt.

Boc-protected (3S,4S)-statine, [(3S,4S)-4-amino3-hydroxy-6- methylheptanoic acid] was produced from the commercially available amino acid, coupled with 2methylalanine using standard peptide synthesis methods and converted to the lithium salt using the method described below. 18.30 g (0.05 mol) of this derivative was stirred in 150 ml dry acetonitrile at room temperature, 5.45 g (0.05 mol) of ethyl chloroformate and 7.0 ml (0.05 mol) of triethylamine were sequentially added with stirring and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The mixture was then stripped to dryness on a rotary evaporator, the residue was triturated with 100 ml of benzene, filtered to remove salts, and the filtrate was again stripped on a rotary evaporator to yield 16.4 g (96%) of crude 2-BOC-(3S,4S)statyl-4,4-dimethyl-5-oxazolone. Analytically pure material was obtained by recrystallization from acetone at -30^{0}C. NMR

($CDCl_3$) - chemical shifts and splitting patterns diagnostic for structure. FTIR (mull): shows a strong azlactone CO band in the 1820 cm^{-1} region.

N-isovaleryl-(S)-2-methylvalyl-(S)-4-methyl-4-isopropyl-5oxazolone.

13.46 g (0.04 mol) of 2-isovaleryl-(S)-2-methylvalyl(S)-2- methyl valine lithium salt, as prepared below, was stirred in 150 ml of dry acetonitrile at room temperature. 4.36 g (0.04 mol) of ethyl chloroformate and 5.6 ml (0.04 mol) of triethylamine were then sequentially added with stirring, and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The mixture was then stripped to dryness on a rotary evaporator, the residue was triturated with 100 ml benzene, filtered to remove salts, and the filtrate was again stripped on a rotary evaporator to yield 12 g (96%) of crude N-isovaleryl(S)-2-methylvalyl-(S)-4-methyl-4-isopropyl-5-oxazolone. Analytically pure material was obtained by recrystallization from acetone at -30^{0}C. NMR ($CDCl_3$): chemical shifts and splitting patterns diagnostic for structure. FTIR (mull): shows strong azlactone CO band in the 1820 cm^{-1} region.

N-isovaleryl-(S)-2-methylvalyl-(S)-2-methyl valine lithium salt.

6.85 g (0.05 mol) of (S)-2-methylvaline lithium salt, prepared from (S)-methyl valine by the method described below, was stirred in 150 ml dry acetonitrile at room temperature and 9.93 g (0.05 mol) of the oxazolone prepared below was added portionwise with cooling. Once addition was complete, the mixture was heated to reflux and held at reflux for 1 hour. The solvent was then stripped *in vacuo* to give a 98% yield of N-isovaleryl-(S)2-

methylvalyl-(S)-2-methyl valine lithium salt. This salt was used directly in the next step.

2-isovaleryl-(S)-4-methyl-4-isopropyl-5-oxazolone.

2-(S)-methylvaline was prepared from (S)-valine by the method described by Kolbe and Barth (Liebigs Ann. Chem. at 1668 (1983)), and was acylated with isovaleryl chloride using standard acylation methods to produce Nisovaleryl-(S)-methylvaline, this was subsequently treated with one equivalent of LiOH in ethanol, followed by removal of the solvent *in vacuo* to yield the N-isovaleryl(S)-methylvaline lithium salt. 22.3 g (0.1 mol) of this Li salt was stirred in 150 ml of dry acetonitrile at room temperature, 10.9 g (0.01 mol) of ethyl chloroformate and 14 ml (0.1 mol) of triethylamine were sequentially added with stirring, and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The mixture was then stripped to dryness on a rotary evaporator, the residue was triturated with 150 ml benzene, filtered to remove salts and the filtrate was again stripped on a rotary evaporator to yield 17.4 g (85%) of crude 2-isovaleryl-(S)-4-methyl-4-isopropyl-5oxazolone. Analytically pure material was obtained by recrystallization from acetone at -30^{0}C. FTIR (mull): shows a strong azlactone CO band in the 1820 cm^{-1} region. NMR ($CDCl_3$): chemical shifts and splitting patterns diagnostic for structure.

EXAMPLE 7.
SYNTHESIS OF A MIMETIC INHIBITOR OF THE HIV PROTEASE

This example teaches the synthesis of a competitive inhibitor for the HIV protease, based on the insertion of a chiral azlactone residue into a strategically important position in the scissile position of the known

substrate, Ac-Ser-Leu-Asn-Phe-Pro-Ile-ValOMe. See, e.g., 33 J. Med. Chem. 1285 (1990) and references cited therein.

0.341 g (1 mmol) of HN-(L)-Pro-(L)-Ile-(L)-ValOMe prepared using standard peptide-synthesis techniques, is dissolved in the minimum amount of DMF. To this mixture is added 0.229 g (1 mmol) 2-acryloyl-(S)-4-ethyl4-benzyl-5-oxazolone described above, and the mixture is stirred at room temperature until the Michael addition reaction has proceeded to completion (as monitored by TLC). 0.393 g (1 mmol) of MeO-D-Ser(Bzl)-D-Leu-D-Asn-NH_2, prepared from the BOC-protected D-amino acids using standard peptide protection and coupling chemistries (see, e.g., J. Med. Chem. 1285 (1990) and references cited therein) is then added and the mixture is heated to 60^0C and stirred at this temperature for an additional 12 hours. The DMF is then removed under high vacuum and the residue is purified by standard C18 reverse-phase chromatography to yield the protected peptide. The sidechain blocking groups are subsequently removed using standard peptide deprotection techniques to yield the product MeO-D-Ser-D-Leu-D-Asn-NH-CO-(S)-Phe-[Me]-NH-COCH2-CH2-L-N-Pro-L-Ile-L-Val-OMe, useful as a competitive inhibitor for the HIV protease.

EXAMPLE 8.
SYNTHESIS OF A MIMETIC INHIBITOR FOR THE HIV PROTEASE

This example teaches the synthesis of another competitive inhibitor for the HIV protease. In this case the phenyl substituent is replaced with a uracil derivative.

0.82 g (1 mmol) of the uracil derivative, whose preparation is described below, is coupled through the free proline carboxylic acid group to 0.244 g (1 mmol) of Ile-

Val-OMe using standard peptide coupling methods. The product is purified by standard C18 reverse-phase chromatography to yield the protected peptide. The Bzl side-chain blocking group is then removed using standard deprotection techniques to yield the product shown above, useful as a competitive inhibitor for the HIV protease.

0.47 g (1 mmol) of the (S)-(S)-prolinevinylazlactone Michael adduct is dissolved in the minimum amount of DMF. 0.488 g (1 mmol) of MeO-D-Ser-(Bzl)-D-LeuD-Asn-NH_2, prepared from the BOC-protected amino acid via standard peptide synthesis techniques (see, e.g., 33 J. Med. Chem. 1285 (1990) and references cited therein) is then added and the mixture is heated to 60^0C and stirred at this temperature for 12 hours. The DMF is then removed under high vacuum to yield 0.95 g of crude product.

2.33 g (5 mmol) of L-proline is dissolved in the minimum amount of DMF, 1.75 g (5 mmol) of racemic uracilfunctionalized azlactone is added and the mixture is stirred at room temperature until the Michael addition reaction proceeds to completion (as monitored by TLC). The DMF is then removed under high vacuum and the diastereomeric mixture is purified by standard normalphase chromatography to give the desired (S)-(S)-Michael adduct.

3.69 g (0.01 mol) racemic N-acryloyl-2-methyl(3'methyluracil)-5'-alanine is stirred with 50 ml of dry acetone and 1.09 (0.01 mol) of ethyl chloroformate was added. 1.4 ml (0.01 mol) of triethylamine is added dropwise over a period of 10 min. and the mixture is stirred at room temperature until the evolution of gas ceases (1.5 hours). The triethylamine hydrochloride is removed by filtration and the cake was slurried with 20 ml of acetone and refiltered. The combined filtrates are concentrated to 50 ml on a rotary evaporator, cooled to 30^0C and the crystallized product collected by filtration and

dried *in vacuo* to yield racemic 4-(2-methyl-5'-[3'methyluracil])4-methyl-2-vinylazlactone.

17.15 g (0.05 mol) of the racemic 2-(3'-methyluracil)5'-methylalanine ethyl ester is added with stirring to a solution of 4.0 g (0.1 mol) sodium hydroxide in 100 ml water. The mixture is stirred until complete solubilization is achieved, and then cooled to 10^0C. 0.05 g 2,6-di-t-butyl-p-cresol is added as a polymerization inhibitor followed by 4.52 g (0.05 mol) acryloyl chloride, which is added dropwise with stirring, keeping the temperature at 10-15^0C with external cooling. To this solution is then added over a 10-min. period 5.7 ml (0.0625 mol) concentrated hydrochloric acid, again keeping the temperature at 15^0C. After the addition is complete, the reaction mixture is stirred for an additional 30 min., cooled to 0_C, and the solid product is collected by filtration, washed well with ice water and pressed firmly with a rubber dam. The resulting wet cake is recrystallized from ethanol/water, and the wet cake is hydrolized with 6N HCL to yield 12.91 g (70%) of racemic N-acryloyl-(3'-methyluracil)-5'-methylalanine.

20.5 g (0.1 mol) of the Schiff base prepared from the ethyl ester of alanine and benzaldehyde according to the method of O'Donnell *et al.* (23 Terahedron Lett. 4259 (1982)) and 17.4 g (0.1 mol) of 3-methyl-5chloromethyl uracil in the mimimum amount of methylene chloride is added dropwise with stirring to a mixture of finely powdered potassium hydroxide and a catalytic amount (0.01 eq) of the phase-transfer reagent $C_6H_5CH_2NEt_3Cl$ in the same solvent at 0^0C. Following addition, the mixture is stirred at 10^0C until the starting material is consumed (approximately 2 hours). An aqueous workup is followed by mild acid hydrolysis of the crude with 1N HCl/Et_2O at 0^0C for 3 hours to yield 29.5 g (86%) of the racemic alpha-methyl amino acid ester.

SYNTHESIS OF 3-METHYL-5-CHLOROMETHYLURACIL

A. 74.08 g (1 mol) of N-methyl urea and 216.2 g (1 mol) of diethylethoxymethylenemalonate are heated together at 122^{0}C for 24 hours, followed by 170_C for 12 hours to yield the 3-methyluracil-5-carboxylic acid ethyl ester in 35% yield, following recrystallization from ethyl acetate.

B. 30 g 3-methyluracil-5-carboxylic acid ethyl ester was saponified with 10% NaOH to give the free acid in 92% yield, after standard work-up and recrystallization from ethyl acetate.

C 20 g of 3-methyluracil-5-carboxylic acid was decarboxylated at 260^{0}C to give a quantitative yield of 3-methyluracil.

D. 3-methyluracil-5-carboxylic acid was treated with HCL and CH_2O using standard chloromethylation conditions to yield 3-methyl-5-chloromethyluracil in 52% yield, following standard work-up and recrystallization from ethyl acetate.

EXAMPLE 9.

PREPARATION OF A CHIRAL CROSSLINKING CONJUGATE MONOMER

4.59 g (0.02 mol) (S)-4-ethyl,4-benzyl-2-vinyl5-oxazolone as prepared in Example 3.3.3 above was added portionwise to a stirred solution of 1.14 g (0.02 mol) allyl amine in 75 ml of methylene chloride cooled to 0^{0}C with an ice bath. After 15 min. the mixture was allowed to warm to room temperature, and was then stirred at room temperature for 4 hours. The solvent was stripped under aspirator vacuum on a rotary evaporator to yield 5.7 g of crude monomer, identified by NMR and FTIR analyses. The product was recrystallized from ethyl acetate to yield pure white crystalline monomer, useful for fabricating croddlinked chiral gels, beads, membranes and composites for chiral separations.

EXAMPLE 10.

SYNTHESIS OF CONJUGATE USEFUL IN ISOLATION AND PURIFICATION OF SEROTONIN-BINDING RECEPTORS

28.6 g (0.1 mol) of sieve-dried octadecane thiol and 13.9 g (0.1 mol) of 2-vinyl-4,4'-dimethylazlactone are mixed in a dry round-bottomed flask equipped with a magnetic stirrer and a drying tube filled with Drierite and cooled in an ice bath. After 1 hour the mixture is allowed to come to room temperature and is held at room temperature for four days. The product is then dissolved in 250 ml of a suitable solvent, the system cooled in an ice bath, and a chilled solution of 17.62 g (0.1 mol) of serotonin in 250 ml of the same solvent is added over a 30-min period. The reaction mixture is allowed to come to room temperature over a 2-hour period and stirred at room temperature for a further 4 hours. The solvent is then removed by freeze drying to yield 60 g of the derivative which is useful as a ligand for the stabilization and isolation of serotonin-binding membrane receptor proteins.

EXAMPLE 11.

SYNTHESIS OF A CONJUGATE USEFUL IN THE ISOLATION AND PURIFICATION OF THE MORPHINE RECEPTOR

To a solution of 0.285 g (0.001 mol) of norcodeine (I) dissolved in 50 ml of the appropriate solvent, such as benzene, is added a solution of 0.139 g (0.001 mol) of 4,4'-dimethylvinylazlactone (II) in 10 ml of the same solvent. The resulting solution is heated to 70^{0}C and held at this temperature for 10 hours. At the end of this time the solvent is removed under vacuum to yield

0.42 g of the Michael adduct (III). 0.21 g (0.0005 mol) of this adduct is added portionwise over a 30 minute period, with stirring, to 0.23 g (0.0005 mol) of lucifer yellow-CH (IV) in 50 ml of a 1:1 mixture of water and an appropriate solvent, such as acetone, adjusted to pH 7.5. at 0^0C under a nitrogen blanket. The reaction mixture is stirred at 0^0C for 1 hour and then allowed to come to room temperature. The mixture is then stirred at room temperature under a nitrogen blanket for 7 days. The solvent is removed under vacuum and the water is removed by freeze drying to give the product (V). (V) is useful as a probe for the study of receptor proteins that bind morphine and its derivatives.

EXAMPLE 12.

SYNTHESIS OF CONJUGATE USEFUL IN THE ISOLATION AND PURIFICATION OF PROTEINS BINDING CIBACRON BLUE

To 4.03 g (0.01 mol) of a stirred solution of thiocholesterol in 100 ml of an appropriate solvent, such as benzene, is added a solution of 1.39 g (0.01 mol) of 2vinyl-4,4'-dimethyl-5-azlactone in 10 ml of the same solvent. The mixture is heated to 70^0C and stirred at this temperature for 4 hours. The solvent is completely removed under vacuum and the product (VI) is redissolved in 200 ml of dimethyl formamide. This solution is cooled in an ice bath and 8.5 g (0.01 mol) of the Cibacron Blue derivative (VII), prepared as described below, dissolved in 250 ml of DMF and 100 ml of triethylamine is added over a 30 min period. The reaction mixture is stirred with cooling for 1 hour, allowed to come to room temperature amd stirred for 12 hours. The mixture is then added to 1 liter of 25% NaCl in water at 0^0C and stirred for 15 min; then 100 ml of 10M hydrochloric acid is added with stirring and cooling, and the blue precipitate is collected by filtration, reslurried in 1 liter of water and refiltered. This extraction

procedure is repeated two more times. The product (VIII) is dried at 60°C in a vacuum oven at 30" of vacuum. (VIII) is useful for inserting and positioning the Cibacron Blue functionality, which is a broadly versatile affinity recognition ligand in cell membranes for the study of transmembrane processes involving proteins that bind to the dye function.

Preparation of Cibacron Blue Derivative (VIII)

40.0 g (0.05 mol) of Cibacron Blue F3 GA is dissolved in 1 liter of DMF at 40°C with stirring. To this solution is added 26.5 g (0.23 mol) of hexamethylene diamine with stirring, followed by 4.0 g (0.05 mol) of pyridine. The reaction mixture is allowed to stir overnight and the pH is adjusted to 2.0 by the addition of 80 ml of 10M hydrochloric acid and 940 g of NaCl. 3.5 liters of water are added to precipitate the modified dye. The mixture is stirred for 1 hour and the dye is collected by filtration. The cake is washed with an additional 3.5 liters of water at pH 2.0 water and dried at 70°C in a vacuum oven at 30" of vacuum to yield 34.0 g of the aminofunctionalized dye (VII).

EXAMPLE 13.

SYNTHESIS OF A PHOTOREACTIVE CONJUGATE USEFUL IN THE ISOLATION AND PURIFICATION OF ß-N-ACETYLGLUCOSAMIDASE

3.63 g (0.01 mol) of 2-acetamido-2-deoxy-1-thiob-D-glucopyranose-3,4,6-triacetate (IX) and 1.39 g of 2vinyl-4,4'-dimethylazlactone are dissolved with stirring in 100 ml of an appropriate solvent, heated to 70°C and held at this temperature with stirring for 12 hours. At the end of this time the mixture is cooled to room temperature and 1.53 g (0.01 mol) of dopamine, dissolved in 50 ml of the same solvent is added, with cooling and stirring, over a 30

min period. The temperature is the allowed to rise to room temperature and the reaction mixture is stirred overnight. The solvent is then removed by freeze drying to produce 6.5 g of the product (X) which is useful for the study of beta-N-acetylglucosamidase and related proteins of similar specificity, since the carbohydrate functionality can bind to these proteins (See 350 Biochim. Biophys. Acta. 437 (1974)). The dopamineconnected catechol functionality is a photographic developer, capable of photographic amplification by means of standard techniques.

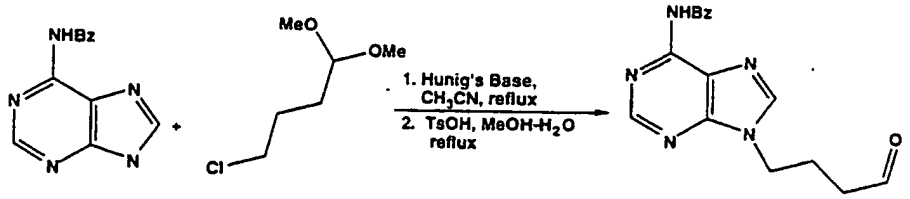
EXAMPLE14.

SYNTHESIS OF A LIGAND OF PROTEIN KINASE

100 mg of the 20-mer cysteine variant, Cys-ThrTyr-Ala-Asp-Phe-Ile-Ala-Ser-Gly-Arg-Thr-Gly-Arg-Arg-AsnAla-Ile-His-Asp, of a protein kinase natural binding peptide ligand PK (5-24) (See, 253 Science 414 (1991)), synthesized by standard peptide synthesis techniques, is shaken with 7 mg of 2-vinyl-4,4'-dimethyl azlactone in 0.5 ml of an appropriate solvent at room temperature for 6 days. At the end of this period 23 mg of Lucifer Yellow CH in 0.5 ml of water is added, and the mixture is shaken at room temperature for 6 hours. The solvents are removed by freeze drying to yield 130 mg of the bifunctional adduct (XI), which is useful as a ligand for competitive evaluation of the binding affinity of competitive ligands for protein kinases and structurally similar proteins.

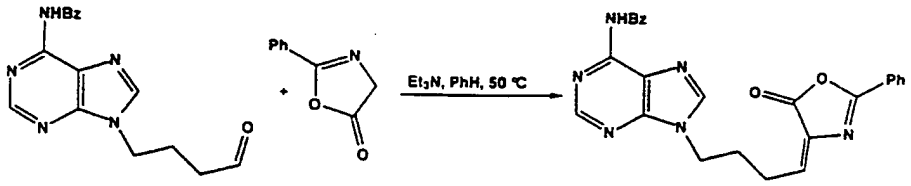
EXAMPLE 15.

SYNTHESIS OF A 4-METHYLENYL-5-OXAZOLONE DERIVED DIMERIC OXAZOLONE OLIGOMER



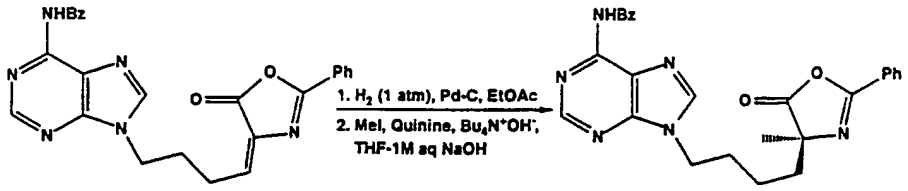
A solution of 6-benzoylamidopurine (23.9 g, 0.10 mole) and diisopropylethylamine (14.22 g, 0.11 mole, 19.16 mL) in acetonitrile (200 mL) is cooled at 0°C while 4-chlorobutryaldehyde (15.26 g, 0.10 mole) is added dropwise. The mixture is stirred at room temperature for 12 hours and the diisopropylethylamine hydrochloride is removed by filtration. The filtrate is concentrated *in vacuo* and recrystallized from ethyl acetate to afford white, powder crystals of the product (22.37 g, 0.063 mole, 63%).

This material is dissolved in methanol (400 mL) to which water (25 mL) and p-toluenesulfonic acid (0.5 g) is added. The mixture is heated at reflux to exhaustion of the acetal. The reaction mixture is concentrated *in vacuo* and the residue partitioned between THF and an aqueous solution of sodium bicarbonate (10 % w/v, 300 mL). The aqueous phase is extracted with THF and the combined orgaincs are dried (sat'd aq NaCl, $MgSO_4$), filtered and concentrated to afford, after recrystallization, 6-benzoylamido-9-(4-oxobutyl)purine (16.38 g, 0.053 mole, 84%).

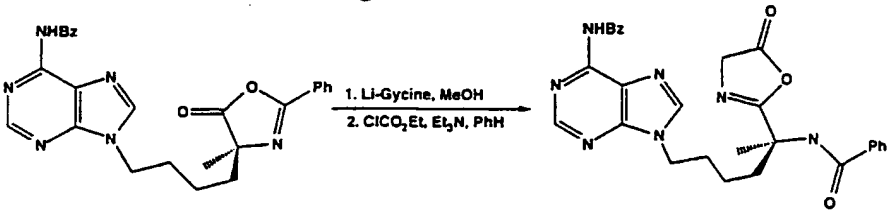


Triethylamine (0.101 g, 1.0 mmol) is added to a solution of 2-phenyl-5-oxazolone (1.61 g, 10 mmol) and 6-

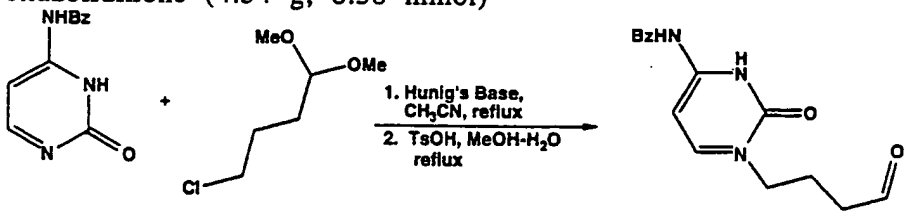
benzoylamido-9-(4-oxobutyl)purine ((3.09 g, 10 mmol) in benzene (20 ml). The resultant mixture is heated to 50^{0}C for 10 minutes and, after cooling to room temperature, the solvent is removed in vacuo. The residual pasty mass is triturated with ethanol to afford a solid which is subsequently recrystallized from ethanol to afford off-white crystals of oxazolidinone-linked benzoyladenine (2.84 g, 63%).



A suspension of the adeninyl oxazolone (4.52 g, 10 mmol) and 10% palladium on carbon (106 mg, 1 mol%) in ethyl acetate (100 mL) is sparged with dry hydrogen gas until the exocyclic methylene is fully reduced (1 equivalent). The catalyst is removed by filtration through a pad of celite and the filtrate is concentrated. The residue is dissolved in tetrahydrofuran (100 mL) and aq NaOH (1.0 M, 100 mL), tetra-*n*-butylammonium hydroxide (0.26 g, 1.0 mmol) and quinine (0.324 g, 1.0 mmol) was added. The mixture is cooled at 0^{0}C while methyl iodide (3.53 g, 25 mmol, 1.55 mL) is added. the mixture is stirred until the oxazolone is exhaustively alkylated. The organic phase is separated and the aqueous phase extracted with ether (2 x 100 mL). The combined organics are dried (sat'd aq NaCl, MgSO) and concentrated to afford a solid (4.98 g) which is chromatographed to afford 4-(4-benzoyladeninylbutyl)-4-methyl-5-oxazolone (3.87 g, 8.27 mmol, 83 %).



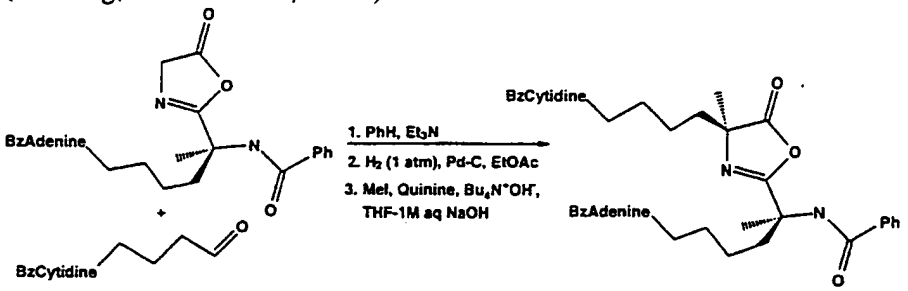
The 4-(4-benzoyladeninylbutyl)-4-methyl-5-oxazolone (3.87 g, 8.27 mmol) is dissolved in methanol (100 mL) and glycine lithium salt (1.01 g, 12.41 mmol)) is added. The mixture is warmed at 50^{0}C for three hours. After the ring-opening reaction is complete water (100 mL) is added and acidified to pH = 5.0 with dil. HCl. The resultant solution is concentrated in vacuo and the solid is dissolved in benzene with the addition of a small percentage of ethyl acetate. The solution is cooled in an ice bath while ethyl chloroformate (1.31 g, 12.41 mmole, 1.18 mL) and triethylamine (1.26 g, 12.41 mmol, 1.32 mL) are added. Following cessation of the gas evolution the salts are removed by suction filtration and the filtrate is concentrated in vacuo. The residue is recrystallized from ethanol to afford a 79% yield of the 2-(5-benzoyladeninyl-2-benzamidoyl-2-methylpentyl)-5-oxazolidinone (4.54 g, 8.58 mmol)



4-Chlorobutryaldehyde (15.26 g, 0.10 mole) is added dropwise to an ice cooled solution of 4-benzoylamidopyrimidinone (21.5 g, 0.10 mole) and diisopropylethylamine (14.22 g, 0.11 mole, 19.16 mL) in acetonitrile (200 mL). The bath is removed and the mixture stirred overnight at room temperature. Removal of the salts by filtration and the solvent in vacuo, followed by recrystallization from ethyl acetate affords the desired product (23.8 g, 0.072 mole, 72%).

To a solution of this acetal dissolved in methanol (400 mL), p-toluenesulfonic acid (0.5 g) and water (25 mL) are added, and the mixture refluxed to exhaustion of the acetal. The reaction mixture is concentrated *in vacuo* and the residue partitioned between THF and an aqueous solution of sodium bicarbonate (10 % w/v, 300 mL). The aqueous phase is

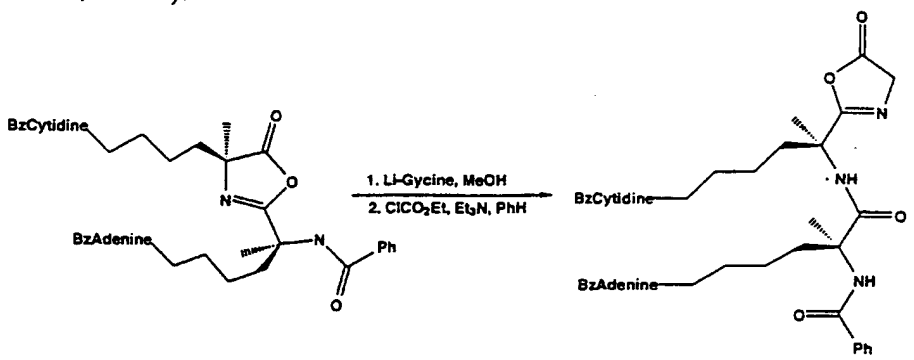
extracted with THF and the combined organics are dried (sat'd aq NaCl, $MgSO_4$), filtered and concentrated to afford, after recrystallization, 4-benzoylamido-1-(4-oxobutyl)pyrimidinone (16.38 g, 0.053 mole, 84%).



A solution of 4-benzoylamido-1-(4-oxobutyl)pyrimidinone (0.57 g, 2.0 mmol), the previously prepared 2-(5-(benzoyladeninyl)-2-benzamidoyl-2-methylpentyl)-5-oxazolidinone (1.06 g, 2.0 mmol), and triethylamine (138 mL, 10 mg, 0.1 mmol as catalyst.) in benzene (20 mL) is warmed at 50^0C for two hours, to exhaustion of the starting materials. Removal of the solvent *in vacuo*, followed by trituration with, then recrystallization from, ethanol affords the product (0.99 g, 1.39 mmol, 70%).

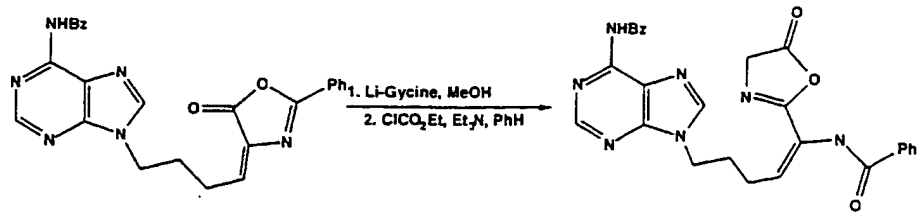
A suspension of the adeninyl oxazolone (0.99 g, 1.39 mmol) and 10% palladium on carbon (15 mg, 1 mol%) in ethyl acetate (15 mL) is sparged with dry hydrogen gas until the exocyclic methylene is fully reduced (1 equivalent). The catalyst is removed by filtration through a pad of celite and the filtrate is concentrated. The residue is dissolved in tetrahydrofuran (15 mL) and aq NaOH (1.0 M, 15 mL), tetra-*n*-butylammonium hydroxide (4 mg, 0.15 mmol) and quinine (45 mg, 0.15 mmol) was added. The mixture is cooledat 0^0C while methyl iodide (0.49 g, 3.5 mmol, 0.22 mL) is added. The mixture is stirred until the oxazolone is exhaustively alkylated. The organic phase is separated and the aqueous phase extracted with ether (2 x 20 mL). The combined organics are dried (sat'd aq NaCl, $MgSO_4$) and concentrated to afford a solid (1.23 g) which is chromatographed to afford 2-(5-(benzoyladeninyl)-2-benzamidoyl-2-methylpentyl)-4-(4-

benzoylcytidinylbutyl)-4-methyl-5-oxazolone (0.87 g, 1.2 mmol, 86 %).

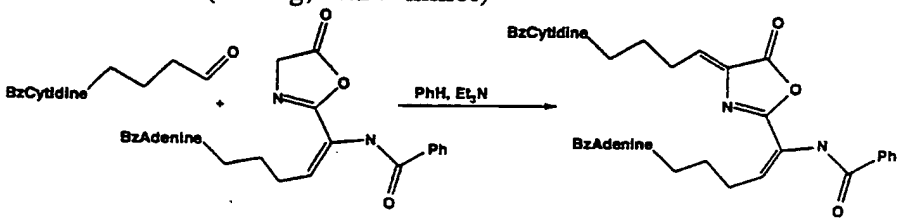


A solution of 2-(5-(benzoyladeninyl)-2-benzamidoyl-2-methylpentyl)-4-(4-benzoylcytidinylbutyl)-4-methyl-5-oxazolone (0.87 g, 1.2 mmol) and glycine lithium salt (150 mg, 1.8 mmol)) in methanol is warmed at 50°C for three hours. After the ring-opening reaction is complete water (10 mL) is added and acidified to pH = 5.0 with dil. HCl. The resultant solution is concentrated in vacuo and the solid is dissolved in benzene with the addition of a small percentage of ethyl acetate. The solution is cooled in an ice bath while ethyl chloroformate (190 mg, 1.8 mmole, 0.17 mL) and triethylamine (183 mg, 1.8 mmol, 0.19 mL) are added. After three hours the salts are removed by suction filtration and the filtrate is concentrated in vacuo. The residue is recrystallized from ethanol to afford a 62% yield of the 2-(2-(5-(benzoyladeninyl)-2-benzamidoyl-2-methylpentanoyl amido)-2-(4-benzoylcytidinyl)-2-methylpentyl)-5-oxazolone (585 mg, 0.74 mmol)

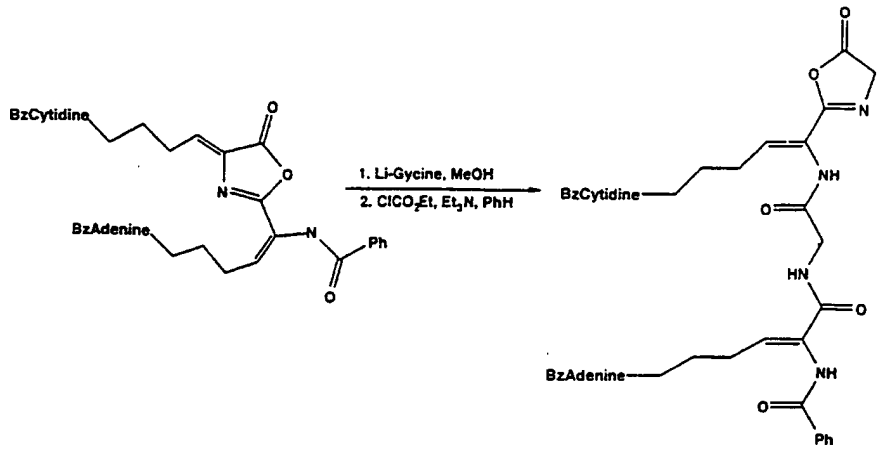
The Erlenmeyer products also may be left unreduced to provide an alternative scaffolding from which to present the recognition groups. Whereas this provides a "flat" structure it will also provide a different spacing and presentation of those groups. Shown below is an experimental sequence to provide the seminal units for such a molecule.



The adeninyl oxazolone (2.84 g, 6.3 mmol) is dissolved in methanol (10 mL) and glycine lithium salt (0.77 g, 9.45 mmol)) is added. The mixture is warmed at 50^{0}C for three hours. After the ring-opening reaction is complete water (100 mL) is added and acidified to pH = 5.0 with dil. HCl. The resultant solution is concentrated in vacuo and the solid is dissolved in benzene with the addition of a small percentage of ethyl acetate. The solution is cooled in an ice bath while ethyl chloroformate (1.03 g, 9.45 mmole, 0.9 mL) and triethylamine (0.96 g, 9.45 mmol, 1.32 mL) are added. Following cessation of the gas evolution the salts are removed by suction filtration and the filtrate is concentrated in vacuo. The residue is recrystallized from ethanol to afford a 67% yield of the oxazolidinone (2.12 g, 4.24 mmol)



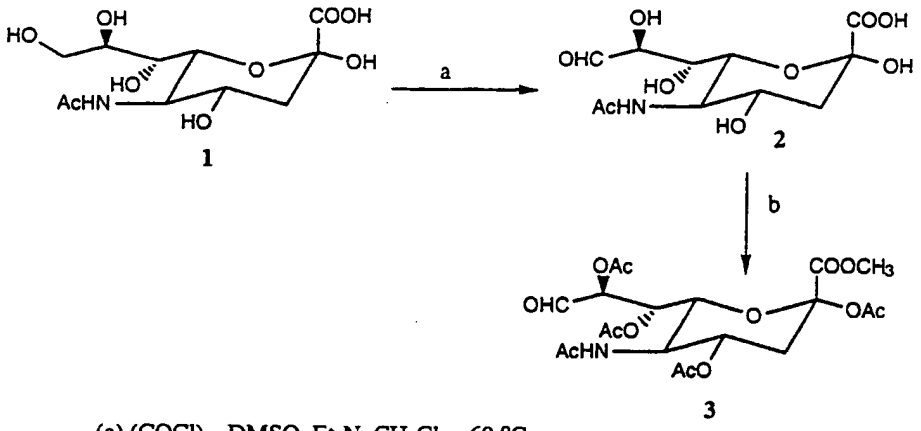
A solution of 4-benzoylamido-1-(4-oxobutyl)pyrimidinone (0.57 g, 2.0 mmol), the previously prepared oxazolidinone-linked benzoyladenine (1.18 g, 2.0 mmol), and triethylamine (138 mL, 10 mg, 0.1 mmol as catalyst.) in benzene (20 mL) is warmed at 50^{0}C for two hours, to exhaustion of the starting materials. Removal of the solvent *in vacuo*, followed by trituration with, then recrystallization from, ethanol affords the product (0.90 g, 1.16 mmol, 58%).



This product is dissolved in methanol (15 mL) and treated with glycine lithium salt (0.14 g, 1.74 mmol)) is added. The mixture is warmed at 50^0C for three hours. After the ring-opening reaction is complete water (10 mL) is added and acidified to pH = 5.0 with dil. HCl. The resultant solution is concentrated in vacuo and the solid is dissolved in benzene with the addition of a small percentage of ethyl acetate. The solution is cooled in an ice bath while ethyl chloroformate (189 mg, 1.74 mmole, 165 mL) and triethylamine (176 mg, 1.74 mmol, 242 mL) are added. Following cessation of the gas evolution the salts are removed by suction filtration and the filtrate is concentrated in vacuo. The residue is recrystallized from ethanol to afford a 51% yield of the oxazolidinone (493 mg, 0.59 mmol)

EXAMPLE 16.

SYNTHESIS OF CARBOHYDRATE MODULE I



(a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -60 °C
(b) Ac_2O, Pyridine, CH_2Cl_2, rt

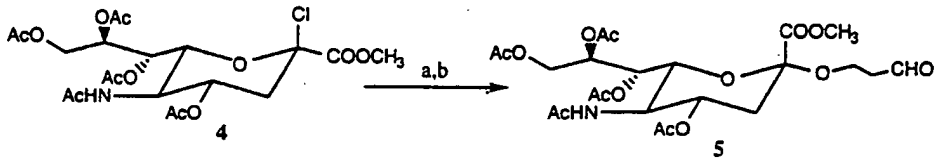
A three neck round-bottom flask is charged with 5 mL of a suitable solvent such as CH_2Cl_2 and 337 mL (3.9 mmol, 1.2 equiv) oxalyl chloride. The solution is stirred and cooled at -60 °C as 460 mL (505 mg, 6.5 mmol, 2 equiv) of DMSO in 5 mL dichloromethane is added dropwise at a rapid rate. After 5 min, compound **1** (1 g, 3.23 mmol, 1.0 equiv) is added dropwise over 10 min maintaining the temperature at -60 °C. After another 15 min, triethylamine (4.5 mL, 32.3 mmol, 10 equiv) is added dropwise while keeping the termperature at -60 °C. Stirring is continued for 5 min, after which time the mixture is allowed to warm to room temperature and water is added. The aqueous layer is seperated and extracted with a somewhat polar solvent such as ethyl acetate. . The organic layers are combined, washed with 1% HCl until it is no longer basic and washed again with saturated sodium chloride and dried over anhydrous magnesium sulfate. The filtered solution is

concentrated by rotary evaporation to otain the aldehyde 2 (900 mg, 91%).

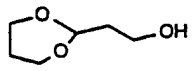
To the aldehyde 2 (500 mg, 1.63 mmol) in pyridine (1.32 mL, 16.3 mmol, 10 equiv) is added acetic anhydride (996 mg, 9.8 mmol, 6 equiv). The reaction mixture is heated on a steam bath for 6 h. The excess pyridine, acetic anhydride and the acetic acid are removed at reduced pressure. The resulting residue is purified by column chromatography to otain the pure product 3 (758 mg, 95%).

EXAMPLE 17.

SYNTHESIS OF CARBOHYDRATE MODULE II



(a) 2-(2-hydroxylethyl)-1,3-dioxane, Ag-Salicylate, THF, rt
(b) aq HCl, THF, rt.



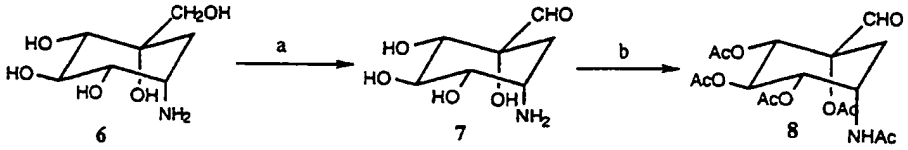
2-(2-hydroxylethyl)-1,3-dioxane

To compound 4 (500 mg, 0.98 mmol) in a suitable solvent such as THF (5 mL) is added Ag-Salicylate (265 mg, 1.08 mmol, 1.1 equiv). After 10 min at room temperature, 2-(2-hydroxylethyl)-1,3-dioxane (130 mg, 0.98 mmol, 1.0 equiv) is added to the mixture. The reaction mixture is stirred at room temperature for 2 h. 1N aqueous HCl (5 mL) is added to the reaction mixture, and the reaction is continued for another 30 min. Water is then added to the reaction. The aqueous

phase is extracted several times with ethyl acetate. The combined organic extract is washed with saturated aqueous NaCl, dried with $MgSO_4$, filtered and concentrated. Purification with column chromatography gives a pure product 5 (483 mg, 90%).

EXAMPLE 18.

SYNTHESIS OF CARBOHYDRATE MODULE III



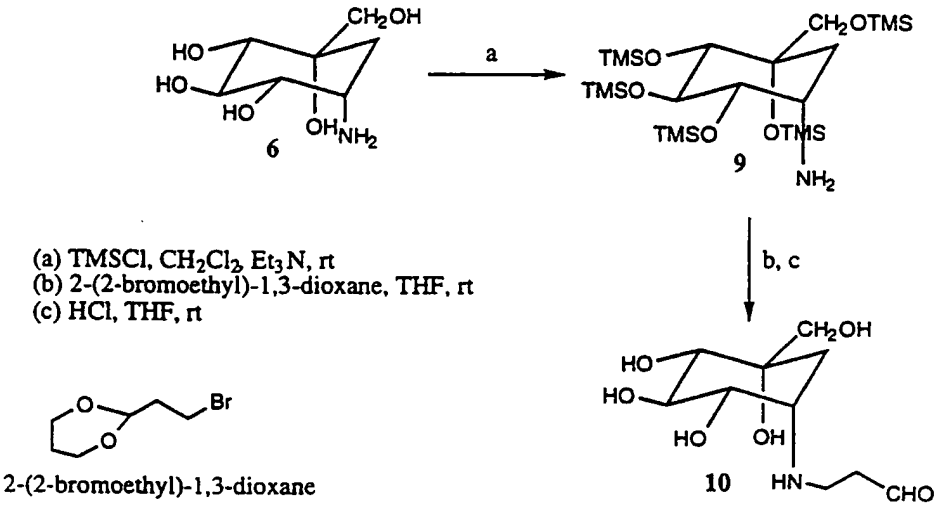
(a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -60 °C
(b) Ac_2O, Pyridine, CH_2Cl_2, rt

A three neck round-bottom flask is charged with 10 mL of a suitable solvent such as CH_2Cl_2 and 540 mL (6.2 mmol, 1.2 equiv) oxalyl chloride. The solution is stirred and cooled at -60 °C as 740 mL (810 mg, 10.4 mmol, 2 equiv) of DMSO in 5 mL dichloromethane is added dropwise at a rapid rate. After 5 min, compound 6 (1 g, 51.8 mmol, 1.0 equiv) is added dropwise over 10 min maintaining the temperature at -60 °C. After another 15 min, triethylamine (7.2 mL, 51.8 mmol, 10 equiv) is added dropwise while keeping the termperature at -60 °C. Stirring is continued for 5 min, after which time the mixture is allowed to warm to room temperature and water is added. The aqueous layer is seperated and extracted with a somewhat polar solvent such as ethyl acetate. The organic layers are combined, washed with 1% HCl until it is no longer basic and washed again with saturated sodium chloride and dried over anhydrous magnesium sulfate. The filtered solution is concentrated by rotary evaporation to otain the aldehyde 7 (890 mg, 90%).

To the aldehyde 7 (800 mg, 4.2 mmol) in pyridine (3.4 mL, 42 mmol, 10 equiv) is added acetic anhydride (3 g, 29.3 mmol, 7 equiv). The reaction is stirred at room temperature for 12 h. The excess pyridine, acetic anhydride and acetic acid are removed at reduced pressure. The residue is purified by column chromatography to give the desired product 8 (1.48 g, 88%).

EXAMPLE 19.

SYNTHESIS OF CARBOHYDRATE MODULE IV

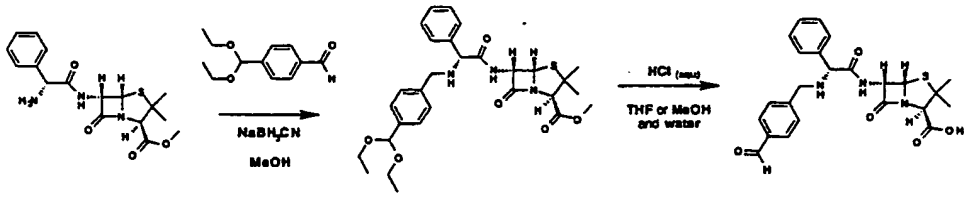


To amine 6 (500 mg, 2.59 mmol) in a suitable solvent such as CH_2Cl_2 (5 mL) is added TMSCl (1.55 g, 14.2 mmol, 5.5 equiv) followed by triethylamine (2.9 mL, 20.7 mmol, 8 equiv). The reaction mixture is stirred at room temperature for 6 h. Water is added to quench the reaction. The organic layer is washed with water and saturared NaCl and dried over anhydrous magnesium sulfate. The filtered solution

is concentrated by rotary evaporation to otain the silylated product **9** (1.3 g, 91%).

To compound **9** (1 g, 1.8 mmol) in a suitable solvent such as THF (8 mL) is added 2-(2-bromoethyl)-1,3-dioxane (387 mg, 1.98 mmol, 1.1 equiv). After 2 h at room temperature, 1N aqueous HCl (10 mL) is added to the reaction mixture and the reaction is continued at room temperature for another 30 min. Water is then added to the reaction. The aqueous phase is extracted several times with ethyl acetate. The combined organic extract is washed with saturated aqueous NaCl, dried with $MgSO_4$, filtered and concentrated. Purification with column chromatography gives a pure product **10** (400 mg, 89%).

EXAMPLE 20.
SYNTHESIS OF DA-AMINO-(*N*-(4-(OXOMETHYL)BENZYL)BENZYL-PENICILLIN:



Synthesis of Dα-Amino-(*N*-(4-(oxomethyl)benzyl)benzyl- penicillin:

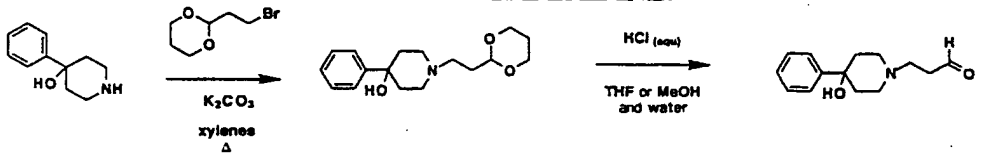
A solution of Dα-Amino-(*N*-(4-(diethoxymethyl)benzyl)benzyl-penicillin, methyl ester (32.6 g, 58.7 mmol), dissolved in an appropriate solvent such as methanol / water or THF / water (100 mL), with an equimolar amount of aqueous 0.5 N HCl is stirred at 50 °C for 4 hours. The solvent is evaporated / lyophilized to afford a solid (29.5 g, 99%). A portion is recrystallized to afford a sample for analysis.

Synthesis of Da-Amino-(*N*-(4-(diethoxymethyl)benzyl)benzyl-penicillin, Methyl ester:

A solution of Da-aminobenzylpenicillin, methyl ester (36.3 g, 99.9 mmol, synthesized from the reaction of D(-)-a-aminobenzylpenicillin in as solution of anhydrous methanol in the presence of a resin-supported super acid, such as Nafion) and 4-(diethoxymethyl)benzaldehyde (21.2 g, 101.8 mmol) in an anhydrous solvent such as THF or methanol (400 mL) under an inert atmosphere such as argon or nitrogen is stirred, typically overnight, until the imine intermediate is formed and the starting reagents consumed as shown by thin layer chromatography (TLC). The reaction mixture is cooled to 0^0C. Sodium cyanoborohydride (7.63 g, 121.4 mmol) is added, and the mixture is stirred at 0^0C for at least 15 min. until the imine intermediate is consumed, as shown by TLC. The solvent is partially evaporated by rotary evaporation. The residual is dissolved in a suitable solvent such as methylene chloride or diethyl ether, extracted with saturated aqueous $NaHCO_3$ (2 x 200 mL) followed by brine (1 x 100 mL), and dried over anhydrous Na_2SO_4. The solvent is removed on a rotary evaporator to yield an off white solid (52.8 g, 95%). A portion is recrystallized to afford a sample for analysis.

EXAMPLE 21.

SYNTHESIS OF 4-HYDROXY-*N*-(2-(1,3-DIOXYL)-ETHYL)-4-PHENYLPIPERIDINE:



Synthesis of 4-Hydroxy-*N*-((1,3-dioxan-2-yl)-ethyl)-4-phenylpiperidine:

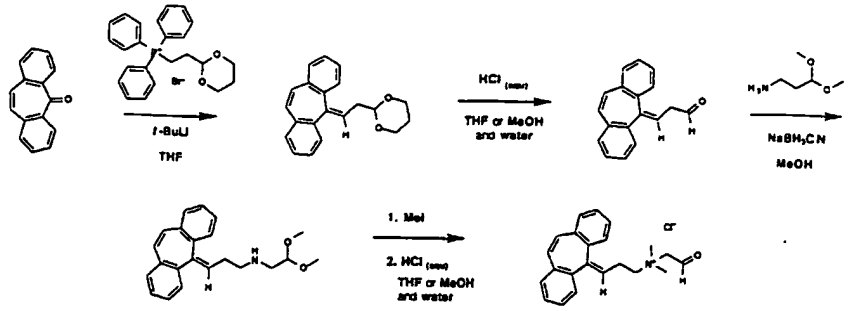
A solution of 4-hydroxy-4-phenylpiperidine (5.00 g, 28.2 mmol) and 2-(2-bromoethyl)-1,3-dioxane (5.53 g, 28.4 mmol) in a suitable solvent such as xylenes or dimethylformamide (DMF) (100 mL) under an inert atmosphere such as argon or nitrogen is gently refluxed, for several hours to overnight, in the presence of K_2CO_3 or another appropriate inorganic base. The reaction mixture is cooled to room temperature, diluted in a suitable solvent such as methylene chloride or diethyl ether, extracted with saturated aqueous $NaHCO_3$ (2 x 100 mL) followed by brine (1 x 100 mL) and dried over Na_2SO_4. The solvent is removed on a rotary evaporator to afford an off white solid (7.31 g, 89%). A portion is recrystallized to yield a sample for analysis.

Synthesis of 4-Hydroxy-*N*-(3-oxopropyl)-4-phenylpiperidine:

A solution of 4-hydroxy-*N*-((1,3-dioxan-2-yl)-ethyl)-4-phenylpiperidine (5.30 g, 18.2 mmol), dissolved in an appropriate solvent such as methanol / water or THF / water (100 mL), with a 1.5 x molar excess of aqueous 0.5 N HCl is stirred at 50 °C for 4 hours. The reaction mixture is diluted in a suitable solvent such as methylene chloride or diethyl ether, extracted with saturated aqueous $NaHCO_3$ (2 x 100 mL) to neutralize the acid, followed by brine (1 x 100 mL) and dried over $MgSO_4$. The solvent is removed on a rotary evaporator to afford an off white solid (4.04 g, 95%). A portion is recrystallized to yield a sample for analysis.

EXAMPLE 22.

SYNTHESIS OF 5*H*-5-((1,3-DIOXAN-2-YL)-2-ETHENYL)-DIBENZO[A,D]CYCLOHEPTENE:



A solution of 2-(1,3-dioxan-2-yl)-ethyltriphenylphosphonium bromide (27.8 g, 60.8 mmol) in a suitable anhydrous solvent, such as THF (300 mL) is cooled at 0 °C while an equimolar amount of a strong base, such as a solution of *n*-butyllithium (2.5 M in hexanes / 25.0 mL) is added dropwise with stirring over a period of 30 minutes. The reaction is stirred at room temperature for another hour or more to ensure the anion formation. A solution of dibenzosuberenone (12.5 g, 60.6 mmol) in an appropriate anhydrous solvent such as THF (100 mL) is added dropwise with stirring over a period of 30 minutes. Stirring continues at 0 °C for another two hours. The reaction is quenched with the addition of water (50 mL). The solvent is partially evaporated by rotary evaporation. The residual is dissolved in a suitable solvent such as methylene chloride or diethyl ether, extracted with saturated aqueous $NaHCO_3$ (2 x 200 mL) followed by brine (1 x 100 mL), and dried over anhydrous Na_2SO_4. The organic solvent is concentrated by rotary evaporation to afford 39 g of a colored oil. The crude material is purified with column chromatography on a suitable stationary phase such as normal phase silica gel and eluted with an appropriate mobile phase such as hexanes / ethyl acetate mixtures, to afford the desired compound (15.7 g, 85%). A portion is repurified to yield a sample for analysis.

Synthesis of 5H-5-(1-oxo-3-propenyl)-dibenzo[a,d]cycloheptene:

A solution of 5*H*-5-((1,3-Dioxan-2-yl)-2-ethenyl)-dibenzo[a,d]cycloheptene (14.3 g, 47.0 mmol), dissolved in an appropriate solvent such as methanol / water or THF / water (100 mL), with a 1.5 x molar excess of aqueous 0.5 N HCl is stirred at 50 °C for 4 hours. The reaction mixture is diluted in a suitable solvent such as methylene chloride or diethyl ether, extracted with saturated aqueous $NaHCO_3$ (2 x 100 mL) to neutralize the acid, followed by brine (1 x 100 mL) and dried over anhydrous $MgSO_4$. The solvent is concentrated by rotary evaporation to afford 13 g of a colored oil. The crude material is purified with column chromatography on a suitable stationary phase such as normal phase silica gel and eluted with an appropriate mobile phase such as hexanes / ethyl acetate, to afford the desired compound (11.1 g, 96%). A portion is repurified to yield a sample for analysis.

SYNTHESIS OF 5*H*-5-((*N*-(2,2-DIMETHOXYETHYL)-1-AMINO-3- PROPENYL)-DIBENZO[A,D]CYCLOHEPTENE:

A solution of 5*H*-5-(1-oxo-3-propenyl)-dibenzo[a,d]-cycloheptene (9.80 g, 39.8 mmol) and aminoacetaldehyde dimethyl acetal (4.21 g, 40.0 mmol) in an anhydrous solvent such as THF or methanol (100 mL) under an inert atmosphere such as argon or nitrogen is stirred, typically overnight, until the imine intermediate is formed and the starting reagents are consumed as shown by thin layer chromatography (TLC). The reaction mixture is cooled to 0 °C. Sodium cyanoborohydride (3.06 g, 48.7 mmol) is added, and the mixture is stirred at 0 °C for at least 30 minutes until the imine intermediate is consumed, as shown by TLC. The solvent is partially evaporated by rotary evaporation. The residual is dissolved in a suitable solvent such as methylene chloride or diethyl ether, extracted with saturated aqueous $NaHCO_3$ (2 x 100 mL) followed by brine (1 x 100 mL), and dried over Na_2SO_4. The solvent is removed on a rotary evaporator to yield a solid

(12.02 g, 90%). A portion is recrystallized to afford a sample for analysis.

SYNTHESIS OF 5*H*-5-((*N*-DIMETHYL-*N*-(2-OXOETHYL)-1-AMINO-3-PROPENYL)-DIBENZO[A,D]CYCLOHEPTENE:

A solution of 5*H*-5-((*N*-(2,2-dimethoxyethyl)-1-amino-3-propenyl)-dibenzo[a,d]cycloheptene (9.71 g, 28.9 mmol) and one equivalent of a non-nucleophilic base such as pyridine, dissolved in an appropriate solvent such as methanol or THF (100 mL) is added iodomethane (8.35 g, 58.8 mmol). The reaction is gently refluxed for several hours to overnight, until the starting material is consumed and the quartenary-substituted amino compound is formed as shown by TLC. A 10 x molar excess of aqueous 1.0 N HCl is added, and stirring continues at 50 °C for 4 hours. The solvent is partially evaporated by rotary evaporation. The residual is dissolved in a suitable solvent such as methylene chloride or diethyl ether, extracted with saturated aqueous $NaHCO_3$ (100 mL) to neutralize the acid, followed by brine (100 mL), then dried over $MgSO_4$. The solvent is removed on a rotary evaporator and vacuum pump to yield a solid (8.16 g, 80%). A portion is recrystallized to afford a sample for analysis.

EXAMPLE 23.

SYNTHESIS OF MATERIALS USEFUL AS COATINGS

This example describes preparation of a coating by a ring-opening reaction followed by Michael-addition.

In the first synthetic step, 8.82 g (0.113 mol) of 95% N-methylethylenediamine were dissolved in 75 ml methylene chloride with stirring and cooled to 0^0C In an ice bath. Then, 13.9 g (0.10 mol) of dimethylvinylazlactone (the starting species illustrated in Eq. 3 with $R_2 = R_3 = CH_3$) pre-cooled to 0^0C were added to the methylene chloride

mixture such that the temperature remained below 5 _C. The solution was then stirred at room temperature. After approximately 15 min a white precipitate began to form. The mixture was stirred for an additional 2 h at 0^0C. A white solid was collected on a Buechner funnel, washed twice with 25 ml methylene chloride and air dried to yield 13.92 g of the ring-opened adduct, identified by nuclear magnetic resonance (NMR) and Fourier transform infrared reflection (FTIR) spectroscopy as follows: NMR ($CDCl_3$): CH_3-N/gem $(CH_3)_2$ ratio 1:2; CH_2 = CH - splitting pattern in 6 ppm region, integration ratios and D_2O exchange experiments diagnostic for structure. FTIR (null): azlactone CO band at 1820 cm^{-1} absent; strong amide bands present in 1670 - 1700 cm^{-1} region.

In the next synthetic step, 6.39 g (0.3 mol) of (I) and 4.17 g (0.3 mol) of dimethylvinylazlactone were dissolved in 50 ml of benzene and heated to 70 _C for 4 h. The flask was cooled to room temperature, stoppered and allowed to stand for 3 days at room temperature. The solvent was then decanted off from the thick oil that had formed. This oil was dissolved in 50 ml acetone and stripped to produce another thick oil. This latter oil was pumped on at 1 torr overnight to yield 3.53 g of a white crystalline solid, identified by NMR and FTIR spectroscopy as follows: NMR: CH_3-N/gem $(CH_3)_2$ ratio 1:4; CH_2 = CH - splitting pattern in 6 ppm region, integration ratios and D_2O exchange experiments diagnostic for structure. FTIR (null): strong azlactone CO band at 1800 cm^{-1}.

In the final synthetic step, 3.5 g (0.01 mol) of (II) and 1.61 g (0.01 mol) of $H_2N(CH_2)_3CH(OC_2H_5)_2$ were dissolved in 50 ml acetone chilled to 0^0C and stirred for 4 h at 0^0C. The solution was allowed to come to room temperature and to stand for 2 days. The resulting yellowish solution was stripped and pumped on at 1 torr at room temperature overnight to produce 5.0 g of a white solid. 4.5 g of this solid were dissolved in hot ethyl acetate,

brought to the cloud point with hot hexane and allowed to crystallize at room temperature overnight. 3.54 g of a white crystalline solid were obtained after collection by filtration and drying in a vacuum oven adjusted for a 30" vacuum at room temperature overnight. The final product was identified by NMR and FTIR spectroscopy as follows: NMR ($CDCl_3$): CH_2 = CH - splitting pattern in 6 ppm region, integration ratios and D_2O exchange experiments diagnostic for structure. FTIR (mull): azlactone CD band at 1820 cm^{-1} absent.

EXAMPLE 24.

PREPARATION OF COATED SILICA SUPPORTS USEFUL IN AFFINITY CHROMATOGRAPHY

This example describes preparation of an affinity coating from compound (III) as prepared in the previous example.

1.76 g (0.0034 mol) of (III) and 0.328 g (0.0032 mol) of n-methylol acrylamide were dissolved in 50 ml methanol, after which 1.11 ml water were added. To this solution were added 5 g of glycidoxypropyl trimethoxysilanefunctionalized silica ("Epoxy Silica"). The mixture was stirred in a rotary at room temperature for 15 min and then stripped, using a bath temperature of 44^{0}C, to a volatiles content of 15% as measured by weight loss (from 25-200^{0}C With a sun gun). The silica, coated as a result of exposure to the mixture of ingredients, was slurried in 50 ml isooctane containing 32.0 mg VAZO-64 (*i.e.*, the polymerization catalyst 2,2'-azobisisobutyronitrile dissolved in 0.5 ml toluene that had been de-aerated with nitrogen. The slurry was then thoroughly de-aerated with nitrogen and subsequently stirred at 70^{0}C for 2 h. The coated silica was then collected by filtration and washed three times in 50 ml methanol, and air dried. Finally, the silica was

heated at 120°C for two hours to cure the coating and yield 5.4 g of coated silica. The silica contained the following attached groups:

1.5 g of the coated silica beads were shaken with 20 ml aqueous HCl (pH = 3.0) for 4 h at room temperature. The course of the reaction was followed by testing for the generation of free aldehyde with ammoniacal silver nitrate (Tollens test). The resulting solid was collected on a Buechner filter, then reslurried and recollected until the wash water was neutral. The silica particles were then air dried to yield 1.25 g of aldehyde packing, the terminal methoxy groups having been replaced with a single aldehyde group as follows:

Repligen Protein A was coupled to the aldehyde packing using the standard conditions given for the attachment of Bovine Serum Albumin in the accompanying instructions (Technical Note No. 4151) from Chromatochem Inc., Missoula, MT.

A one-cm glass column was packed with the ProteinA functionalized material and loaded with human IgG from PBS buffer (pH = 7.4) at a flow rate of 1.6 ml/min. The IgG was eluted in 0.01M NaOAc (pH = 3.0). The IgG was then collected and the amount measured spectrophotometrically using standard calibration curves. The measured capacity of the packing was 12 mg IgG per ml of column volume.

EXAMPLE 25.

FUNCTIONALIZATION OF AZLACTONE-CONTAINING POLYMERS

It is possible to procure existing azlactone functionalized polymeric surfaces (e.g., as described in U.S. Patent No. 4,737,560) and to functionalize them according to the steps outlined above. For example, by using

successive reactions with dinucleophilic species of the form HNu^1-Z-Nu^2H and suitable azlactones, a surface of the form

(SURFACE)-(X)-Az,

where X is a linker and Az stands for axlactone, can be transformed into the species
(SURFACE)-(X)-$CONHC(CH_3)_2CONu^1(Z)Nu^2CH_2CH_2$-Az

which may be linked, if desired, to a biomolecule to form the following conjugate:

(SURFACE)
\
(X)-
$CONHC(CH_3)_2CONu^1(Z)Nu^2CH_2CH_2CONHC(CH_3)_2$COBiomolecule

A suitable experimental procedure is as follows. The azlactone-functional support is slurried in a suitable solvent, such as $CHCl_3$, and cooled to 0^0C. An amount of the bifunctional nucleophile equivalent on a molar basis to the total number of surface azlactone groups present, is dissolved in the same solvent and added with shaking. The mixture is then shaken at 0^0C for 6 hours, allowed to come to room temperature, and shaken at room temperature overnight. The support is collected by filtration, washed with fresh solvent, re-slurried in an appropriate solvent and one equivalent of vinylazlactone, dissolved in the same solvent, is added thereto. The mixture is then shaken, heated to 70^0C and held at this temperature for 12 hours. At the end of this time, the mixture is cooled and the support collected by filtration. The support is then washed thoroughly with fresh solvent and dried *in vacuo*.

EXAMPLE 26.

PREPARATION OF A SUPPORT USEFUL IN THE PURIFICATION OF HUMAN IGG FROM SERUM

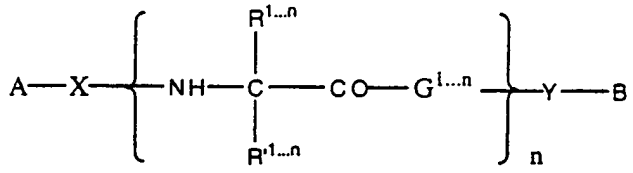
The functional beads prepared as above are suspended in pH 7.5 aqueous phosphate buffer. A solution of protein A (Repligen) in 10 mM phosphate buffer (pH 7.0) and at a concentration of 10 mg/900 Ul is added, and the mixture is then gently shaken at room temperature for 3 hours. The beads are concentrated by centrifugation, the supernate decanted off and the beads washed five times with pH 7.5 aqueous phosphate buffer. The beads are then loaded into a 0.46 cm inner-diameter glass column and used to purify human IgG from serum using standard affinity purification techniques.

It should be apparent to those skilled in the art that other compositions and processes for preparing the compositions not specifically disclosed in the instant specification are, nevertheless, contemplated thereby. Such other compositions and processes are considered to be within the scope and spirit of the present invention. hence, the invention should not be limited by the description of the specific embodiments disclosed herein but only by the following claims.

THE CLAIMS

What is claimed is:

1. A composition having the structure:



wherein:

a. A and B are the same or different, and each is a chemical bond; hydrogen; an electrophilic group; a nucleophilic group; R; an amino acid derivative; a nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component, wherein A and B are optionally connected to each other or to other structures and R is as defined below;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfer, oxygen or combinations thereof;

c. R and R' are the same or different and each is selected from the group consisting of A, B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic aryl and substituted or heterocyclic derivatives thereof,

wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

provided that, (1) if n is 1, and X and Y are chemical bonds, A and B are different and one is other than a chemical bond, H or R, and A and B each is other than an amino acid residue or a peptide; (2) if n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group and G-B is not an amino acid residue or a peptide; (3) if n is 1 and X, Y, and G each is a chemical bond, A and B each is other than a chemical bond, an amino acid residue or a peptide; and (4) if n is 1, either X or A has to include a CO group for direct connection to the NH group.

2. The composition of claim 1 wherein G is chemical bond or the ring-opening reaction product of a nucleophilic group and an oxazolone and $n > 2$.

3. The composition of claim 1 wherein at least one of R and R' includes a hydroxyl containing substituent.

4. The composition of claim 1 wherein X is a carbonyl group.

5. The composition of claim 1 wherein G includes a NH, OH or SH terminal group for connection to the carbonyl group.

6. The composition of claim 1 wherein G is a chemical bond and Y is a compound which includes a NH, OH or SH terminal group.

7. The composition of claim 1 wherein G is a chemical bond, Y is an oxygen atom and B is a hydrogen.

8. The composition of claim 1 wherein G includes at least one of an aromatic ring, a heterocyclic ring, a carbocyclic moiety, an alkyl group or a substituted derivative thereof.

9. The composition of claim 1 wherein A and B are the same.

10. The composition of claim 1 wherein R and R' are different so that the composition is chiral.

11. The composition of claim 1 wherein at least one of A and B is a terminal-structural moiety of formula T-U, wherein:

a. U is selected from the group consisting of aliphatic chains having from 2 to 6 carbon atoms, substituted or unsubstituted aryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocyclic rings; and

b. T is selected from the group consisting of OH NH_2, SH, $(CH_3)_3N^+$, $-SO^-_3$, COO^-, CH_3, H, and phenyl.

12. The composition of claim 11 wherein at least one of A and B is $HO\text{-}CH_2\text{-}(CHOH)_n$ where n is an integer.

13. The composition of claim 1 wherein A and B are part of the same cyclic moiety.

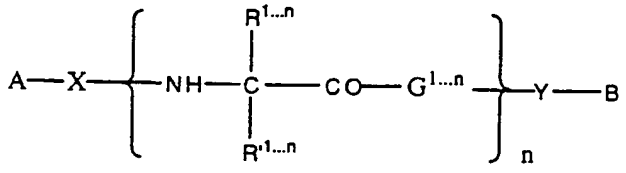
14. The composition of claim 1 wherein n is 1 and G includes a NH, OH or SH terminal group for connection to the carbonyl group.

15. The composition of matter of claim 14 wherein G is a group containing the atom of the nucleophile used in the ring-opening reaction of an oxazolone.

16. The composition of claim 14 wherein R and R' are different so that the composition is chiral.

17. The composition of claim 1 wherein R and R' are different, X is a chemical bond and A is nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component.

18. A peptide mimetic having the structure



wherein:

a. A and B are the same or different, and at least one is an amino acid derivative of the form $(AA)_m$, wherein AA is a natural or synthetic amino acid residue and m is an integer, and A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfer, oxygen or combinations thereof;

c. R and R' are the same or different and each is selected from the group consisting of A, B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

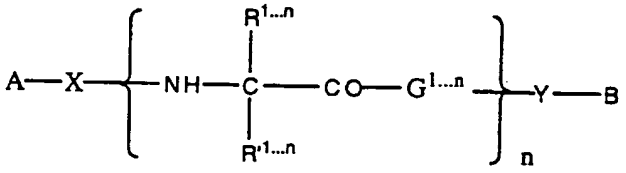
provided that, when (1) n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group and G-B is not an amino acid residue or a peptide; (2) if n is 1 and X, Y, and G each is a chemical bond, A and B each is other than a chemical bond, an amino acid residue or a peptide; and (3) if n is 1, either X or A has to

include a CO group for direct connection to the NH group.

19. The composition of claim 1 wherein G is (1) Nu^1-Y-P where Nu^1 is a nucleophilic group, Y is as defined above and P is a reactive group optionally containing a protective group; or (2) as α–α-di-substituted amino acid residue.

20. The composition of claim 19 wherein P is a nucleophilic group optionally containing a protective group.

21. A nucleotide mimetic having the structure:



wherein:

a. A and B are the same or different, and at least one is a nucleotide derivative, wherein A and B are optionally connected to each other or to other structures;

b. X and A are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfer, oxygen or combinations thereof;

c. R and R' are the same or different and each is selected from the group consisting of A, B,

cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

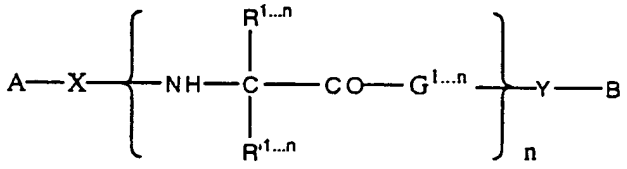
d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

provided that, when n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group.

22. The nucleotide mimetic of claim 21 wherein A is a nucleotide derivative of the form $(NUCL)_l$, wherein l is an integer, such that $(NUCL)_l$ is a natural or synthetic nucleotides when l=1, a nucleotide probes when l=2-25 and an oligonucleotides when l>25 including both deoxyribose (DNA) and ribose (RNA) variants.

23. A carbohydrate mimetic having the structure:



wherein:

a. A and B are the same or different, and at least one is a carbohydrate derivative; wherein A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfer, oxygen, or combinations thereof;

c. R and R' are the same or different and each is selected from the group consisting of A, B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

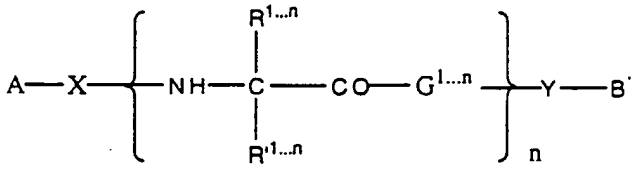
d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

provided that, when n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group.

24. The carboyhdrate mimetic of claim 23 wherein A and B each is a natural carbohydrate, a synthetic carbohydrate residue or derivative thereof or a related organic acid thereof.

25. A pharmaceutical compound having the structure:



wherein:

a. A and B are the same or different, and at least one is an organic structural motif; wherein A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfer, oxygen or combinations thereof;

c. R and R' are the same or different and each is selected from the group consisting of A, B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

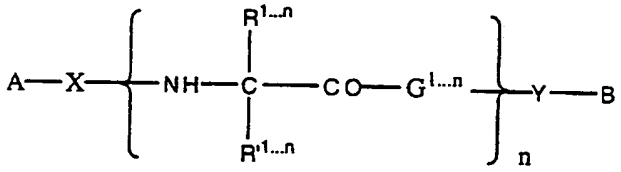
d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

provided that, when n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group.

26. The pharmaceutical compound of claim 25 wherein the structural motif of the organic compound is obtained from a pharmaceutical compound or a pharmacophore or metabolite thereof and has specific binding properties to ligands.

27. A reporter compound having the structure:



wherein:

a. A and B are the same or different, and at least one is a reporter element; wherein A and B are optionally connected to each other or oto other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfer, oxygen or combinations thereof;

c. R and R' are the same or different and each is selected from the group consisting of A, B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic aryl

and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

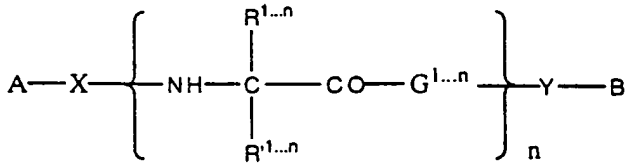
e. $n \geq 1$;

provided that, when n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group.

28. The reporter compound of claim 27 wherein the reporter element is a natural or synthetic dye or a photographically active residues which possesses reactive groups which may be synthetically incorporated into the oxazolone structure or reaction scheme and may be attached through the groups without adversely interfering with the reporting functionality of the group.

29. The reporter compound of claim 28 wherein the reactive group is amino, thio, hydroxy, carboxylic acid, acid chloride, isocyanate alkyl halide, aryl halide or an oxirane group.

30. A polymerizable compound having the structure:



wherein:

a. A and B are the same or different, and at least one is an organic moiety containing a polymerizable group; wherein A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitorgen, sulfer, oxygen or combinations thereof;

c. R and R' are the same or different and each is selected from the group consisting of A, B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

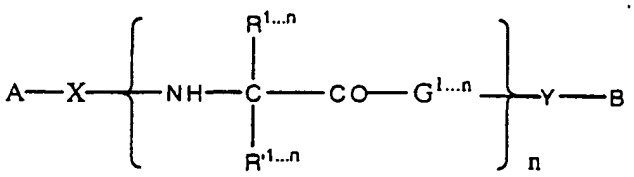
e. $n \geq 1$;

provided that, when n is 1 and Y is a chemical bond, G includes a NH< OH or SH terminal group for connection to the carbonyl group.

31. The polymerizable compound of claim 30 wherein the polymerizable group of the organic moiety is

a vinyl group, oxirane group, carboxylic acid, acid chloride, ester, amide, lactone or lactam.

32. A substrate having the structure:



wherein:

a. A and B are the same or different, and at least one is a macromolecular component, wherein A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is selected from the group consisting of A, B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

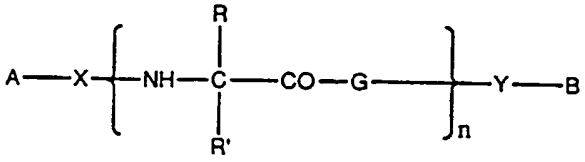
provided that, when n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group.

33. The substrate of claim 32 wherein the macromolecular component is a surface or structures which is attached to the oxazolone module via a reactive group in a manner where the binding of the attached species to a ligand-receptor molecule is not adversely affected and the interactive activity of the attached functionality is determined or limited by the macromolecule.

34. The substrate of claim 32 wherein the macromolecule component has a molecular weight of at least about 1000 Daltons.

35. The substrate of claim 32 wherein the molecular component is in the form of a ceramic particle, a nanoparticle, a latex particle, a porous or non-porous beads, a membrane, a gel, a macroscopic surface or a functionalized or coated version or composite thereof.

36. A composition having the structure:



wherein:

a. A is a chemical bond; hydrogen; an electrophilic group; a nucleophilic group; R; an amino acid derivative; a nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component, wherein R is as defined below;

b. Y is a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached; and

d. q = 0 or 1.

37. The composition of claim 36 wherein Y includes at least one nucleophilic species which includes a nitrogen, oxygen or sulfur group attached to a $-(CG_2)_n-$ group where n is 1-2, and R and R' are the same or different and each is hydrogen, or an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group, or a carbocyclic or heterocyclic ring.

38. The composition of claim 36 wherein Y is a chemical bond and q = O or Y is;

$$(RING) - (CH_2)_n$$

where n=0-4 and (RING) designates a disubstituted phenyl ring or a substituted or unsubstituted aromatic, heterocyclic or alicyclic ring having 6-20 carbons, wherein A is a protecting group when Y contains a terminus which can react with the oxazolone ring.

39. A method of synthesizing a compound of the formula:
$B\text{-}Y\text{-}(CO\text{-}CRR'\text{-}NH)_n\text{-}H$

wherein

a. B is an amino acid derivative; a nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component;

b. Y represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is selected from the group consisting of A, B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 2$;

which method comprises the steps of:

providing a first amino-blocked oxazolone of the formula:

B_1-NH-CRR'-oxazolone ring with R and R'

reacting the first amino-blocked oxazolone under conditions that promote ring-opening with a compound that includes B and has a ring opening reactive moiety to form an amino-blocked ring-opened adduct; and

deblocking the adduct by removing the amino-blocking group.

40. The method of claim 39 which further comprises:

providing a free amino group on the deblocked adduct;

providing a second amino-blocked oxazolone;

reacting the free amino group of the adduct with the second amino-blocked oxazolone to form a second adduct; and

repeating the preceding steps, if necessary, to provide the desired structure of the composition.

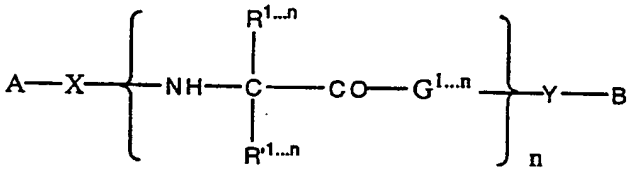
41. The method of claim 39 which further comprises selecting the compound that is to react with the first oxazolone to include an amine, hydroxyl or sulfhydryl group to promote the ring opening; and

selecting R and R' to be different so that a chiral molecule is obtained.

42. The method of claim 39 wherein the starting materials used are achiral or not enantiomerically pure.

43. The method of claim 39 further comprising the step of reacting the free amino group of the oxazolone with a carboxyl terminus of a peptide.

44. A method of synthesizing a compound of the form:



wherein

a. A and B are the same or different, and each is an amino acid derivative; a nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component, wherein A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

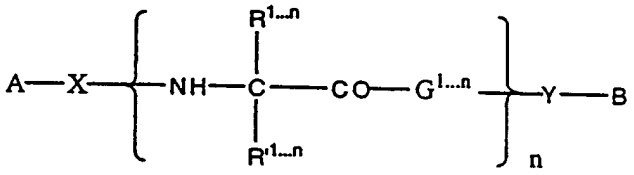
c. R and R' are the same or different and each is selected from the group consisting of A, B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

wherein the method comprises the steps of:

providing an oxazolone of the formula:



where A, R, R' and Y are as defined above and q=0 or 1; and

reacting the oxazolone under conditions that promote ring-opening with a compound that includes B and has a ring opening reactive moiety to form a ring-opened adduct.

45. The method of claim 44 which further comprises: cxarrying out an appropriate subsequent reaction on the previous ring-opened product, wherein the subsequent reaction is:

1) in the case where G is a chemical bond, cyclizing the terminal α,α-asymmetrically disubstituted amino acid to form a terminal azlactone ring;

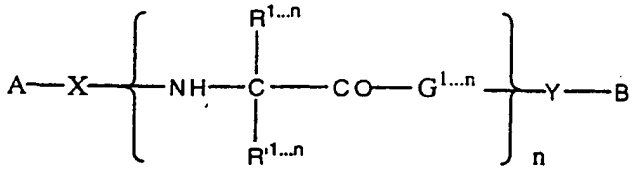
2) in the case where G is -Nu-Z where Nu is a group which includes sulfur, nitrogen or oxygen and ZX includes a carboxylk carboxyl, isocyanate or acid halide terminus, adding the terminus of Z to the amino terminus of an α,α'-asymmetrically disubustituted amino acid and then cyclizing the resulting amino acid to form a terminal oxazolone ring; or

3) in the case where G is $-Nu_1-Z-Nu_2-CH_2CH_2-CO-$ where Nu^1 and Nu^2 each is a group which includes sulfur, nitrogen or oxygen and Z is a connecting group, reacting the Nu2 terminus with the vinyl group of a 4,4'-asymmetrically disubstituted 2-vinyl oxazolone under conditions that promote a Michael addition reaction to form a terminal oxazolone ring;

repeating the preceding steps, if necessary, to provide the desired structure of the composition; and

reacting the terminal oxazolone ring with a species of the form G^n-B-YH to form the composition.

46. A method of synthesizing a compound of the formula:



wherein

a. A and B are the same or different, and each is an amino acid derivative; a nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component, wherein A and B are optionally connected to each other or to other structures;

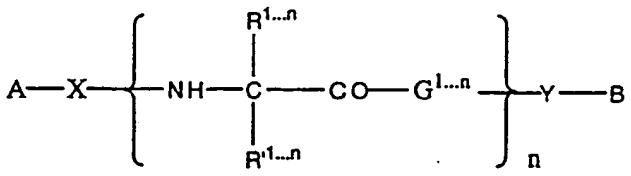
b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is selected from the group consisting of A, B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond;

wherein the method comprises the steps of:

reacting an amino acid of the form wherein R and R' are as recited above, with a carboxylic acid, an acid halide or an oxazolone to form an adduct of the formula:



cyclizing the adduct to form an oxazolone;

reacting the oxazolone with a bifunctional species of the form HX-Z-Y, wherein HX includes an amine, hydroxyl or sulfhydryl group and Y contains a reacxtive group capable of bonding with species B; and

reacting the resultant product with species B.

47. The method of claim 46 wherein the peptide sequence is chiral.

48. A method of synthesizing a compound containing a peptide sequence which comprises the steps of:

providing a substrate bound, via a CO group, to the amino terminus of an α,α'-disubstituted chiral amino acid;

cyclizing the amino acid into an oxazolone;

reacting the oxazolone with an alkali-metal salt of a second α,α'-disubstituted chiral amino acid to form a bound dipeptide salt;

cyclizing the second α,α'-disubstituted chiral amino acid;

repeating steps (c) and (d), if necessary, to form the desired peptide sequence.

49. The method of claim 48 wherein the structure of the composition is not obtained chirally pure.

50. The method of claim 49 which further comprises the step of releasing the composition from the substrate.

51. The method of claim 48 which furher comprises the step of reacting a cyclized oxazolone intermediate with a species containing a reactive moiety of an amine, hydroxyl or sulfhydryl group

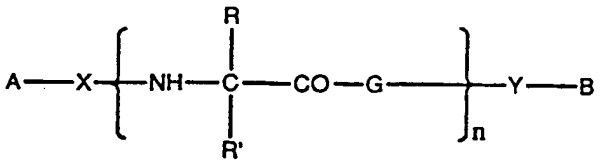
52. The method of claim 48 wherein Y-Z-B is an aminimide.

53. The method of claim 48 wherein the peptide sequence is chiral.

54. A compound produced by the method of any one of claims 39 to 53.

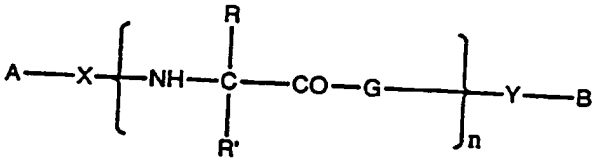
55. A method of making a polymer having a particular water solubility comprising the steps of:

choosing a first monomer having the formula



wherein R and R' are the same or different and are chosen from those organic moieties exhibiting hydrophobicity;

choosing a second monomer having the formula



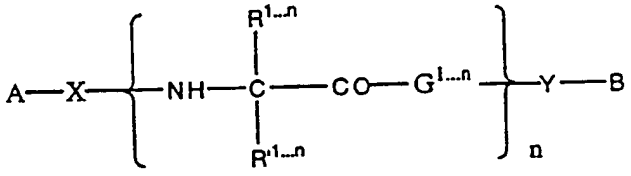
wherein R and R' are the same or different and are chosen from those organic moieties exhibiting hydrophilicity; and

reacting said monomers to provide an effective amount of each monomer in a developing polymer chain until a polymer having the desired water solubility is created.

56. A method according to claim 55 wherein the hydrophobic organic moieties include those which do not have carboxyl, amino or ester functionality.

57. A method according to claim 55 wherein the hydrophilic moieties include those which do not have carboxyl, amino or ester functionality.

58. A method of preparing a synthetic compound to mimic or complement the structure of a biologically active compound or material which comprises synthesizing a compound of the formula:



wherein

A is a chemical bond; hydrogen; an electrophilic group; a nucleophilic group; R'; an amino acid derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component, wherein A and B are optionally connected to each other or to other structures and R is as defined below;

X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

R and R' are the same or different and each is selected from the group consisting of A, B, isomers of A and B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic, aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

G is a chemical bond or a connecting group that includes a terminal carbon atom for attachment to the

quaternary nitrogen and G may be different in adjacent n units; and

$$n > 1.$$

59. A method according to claim 58 wherein said compound is a pharmacaphore.

60. A method according to claim 58 wherein said compound is a peptide mimetic.

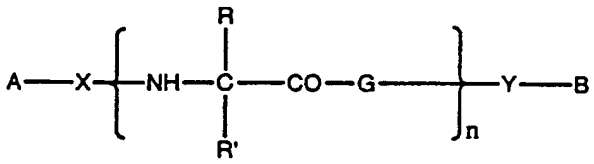
61. A method according to claim 58 wherein said compound is a nucleotide mimetic.

62. A method according to claim 58 wherein said compound is a carbohydrate mimetic.

63. A method according to claim 58 wherein said compound is a reporter compound.

64. A method of preparing a combinatorial library which comprises:

preparing a compound having the formula;



wherein

A is a chemical bond; hydrogen; an electrophilic group; a nucleophilic group; R'; an amino acid derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component,

A is a chemical bond; hydrogen; an electrophilic group; a nucleophilic group; R'; an amino acid derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component, wherein A and B are optionally connected to each other or to other structures and R is as defined below;

X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

R and R' are the same or different and each is selected from the group consisting of A, B, isomers of A and B, cyano, nitro, halogen, oxygen, hydroxy, alkoxy, thio, straight or branched chain alkyl, carbocyclic, aryl and substituted or heterocyclic derivatives thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

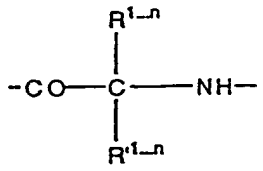
G is a chemical bond or a connecting group that includes a terminal carbon atom for attachment to the quaternary nitrogen and G may be different in adjacent n units; and

$n \geq 1$; and

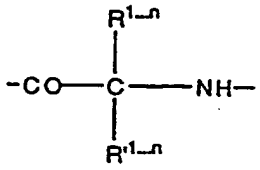
contacting the separator compound with the plurality of compounds; and

differentiating the second compound from plurality of compounds.

65. The method of claim 55 or 64 wherein G is an oxazolone isomer having the formula;



66. The composition of claims 1, 11, 18, 21, 23, 24, 25, 27, 30, 32, 36, 44, 46, 55, 58, or 64 wherein G is an oxazolone isomer having the formula;



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/12591

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/00
US CL : 514/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS Online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	L. Stryer, "Biochemistry", 2nd Edition, published 1981 by W.H. Freeman & Co. (NY), pages 14-17, see entire document.	1, 2, 6, 7, 18 ---------- 3, 8, 10, 11, 14, 16, 17, 19, 20, 25-27, 30-32 ---------- 4, 5, 9, 12, 13, 15, 28, 29, 33-35, 37, 38.
A	Science, Vol 258, issued 27 November 1992, J. C. Hanvey et al., "Antisense and Antigene Properties of Peptide Nucleic Acids", pages 1481-1485.	21-22

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
20 JUNE 1994

Date of mailing of the international search report
JUN 24 1994

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INTERNATIONAL SEARCH REPORT

Int. national application No.
PCT/US93/12591

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 5,219,731 (SIH) 15 June 1993, see entire document.	39-63 and 66
A	US, A, 5,182,366 (HUEBNER ET AL) 26 January 1993, see entire document.	64-65